IN THE UNITED STATES PATENT AND TRADEMARK OFFICE #12

In Re	:	U.S. Patent No. 4,883,812
Issued	:	November 28, 1989
Inventors	:	Salvador Moncada
Assignee	:	Burroughs Wellcome Co.
For	:	TREATMENT OF HYPERTENSION USING PROSTACYCLIN

Commissioner of Patent and Trademarks
Box Patent Extension
Washington, DC 20231

APPLICATION FOR PATENT TERM EXTENSION UNDER 35 U.S.C. 156

P 30021 11/21/95 4883812 02-4857 030 111 1,060.00CH

Sir:

Applicant, Glaxo Wellcome Inc., a corporation of the State of North Carolina and successor in interest to Burroughs Wellcome Co. by virtue of merger and change of name, represents pursuant to 35 U.S.C. 156(d)(1), that it is the record owner and assignee of the entire interest in and to Letters Patent of the United States of America No. 4,883,812 granted to Salvador Moncado on November 28, 1989 for TREATMENT OF HYPERTENSION USING PROSTACYCLIN by virtue of an assignment to Burroughs Wellcome Co. recorded in the United States Patent and Trademark Office on December 7, 1977, Reel 3479, Frames 683-686. A true copy of said assignment and Certificate of Merger / Change of Name are attached hereto as EXHIBITS 1 & 2, respectively.

Applicant further represents, pursuant to 37 C.F.R. 1.785(d), that it is the holder of the regulatory approval granted by the Food and Drug Administration ("FDA") for FLOLAN® (epoprostenol sodium) for Injection (hereinafter, "FLOLAN® Injection"). See EXHIBIT 4.

Applicant presents this Application for Extension of Patent Term under 35 U.S.C 156 according to the format set forth in 37 C.F.R. 1.740(a).

Applicant hereby submits this application, in the alternative with an application for extension of U.S. Patent 4,338,325, both in respect of FLOLAN® Injection, duly electing U.S. Patent 4,883,812 to be extended and in the alternative U.S. Patent 4,338,325 pursuant to 37 C.F.R. 1.785(b). Applicant makes known that the present election is not intended to waive any right of appeal should its election or alternative be denied a term extension.

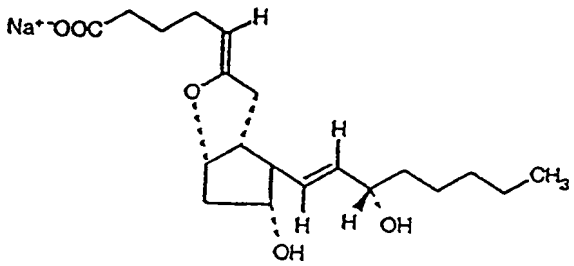
(1) This application for extension is based upon the regulatory review period before the FDA of Applicant's approved product, FLOLAN® Injection. The only active ingredient in FLOLAN® Injection is epoprostenol sodium. A copy of the labeling approved by the FDA as part of New Drug Application ("NDA") 20-444 for the approved product is attached hereto as EXHIBIT 5. Identification of the approved product, FLOLAN® Injection, is provided as follows:

Chemical Name: Epoprostenol is (5*Z*,9,11,13*E*,15*S*)-6,9-epoxy-11,15-dihydroxyprosta-5,13-dien-1-oic acid.

The active ingredient is epoprostenol sodium.
(as per approved labeling, see EXHIBIT 5)

Molecular Formula: $C_{20}H_{31}NaO_5$

Structural Formula:



Molecular Weight: 374.45 Daltons

Description: FLOLAN® (epoprostenol sodium) for Injection is a sterile sodium salt formulated for intravenous administration.

FLOLAN® is a white to off-white powder that must be reconstituted with STERILE DILUENT for FLOLAN® .

After being reconstituted, each vial of FLOLAN® contains epoprostenol sodium equivalent to either 0.5 mg or 1.5 mg epoprostenol, 3.76 mg glycine, 2.93 mg sodium chloride, 50 mg mannitol and Water for Injection, USP. Sodium hydroxide may have been added to adjust the pH between 10.2 and 10.8. See EXHIBIT 5.

(2) The approved product, FLOLAN® Injection, was subject to regulatory review under Federal Food, Drug and Cosmetic Act, section 505 (21 U.S.C. 355).

(3) FLOLAN® Injection received permission for commercial marketing or use under section 505 of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 355) on September 20, 1995. See EXHIBIT 4.

(4) Epoprostenol sodium, the only active ingredient in FLOLAN® Injection, has not been previously approved for commercial marketing under the Federal Food Drug and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act.

(5) This application for extension of patent term under 35 U.S.C. 156 is being submitted within the permitted 60 day period, which will expire on November 18, 1995.

(6) The complete identification of the patent for which extension of term is being sought is as follows:

U.S. Patent	:	4,883,812
For	:	TREATMENT OF HYPERTENSION USING PROSTACYCLIN
Inventors	:	Salvador Moncado
Assignee	:	Burroughs Wellcome Co.
Issue Date	:	November 28, 1989
Expiration Date (as terminally disclaimed against U.S. 4,539,333)	:	September 3, 2002

(7) A complete copy of the patent identified in paragraph (6) above is appended hereto as EXHIBIT 6.

(8) (a) Copies of the receipts of all maintenance fee payments made with respect to U.S. Patent 4,883,812 are attached hereto as EXHIBIT 3.

(b) A copy of the Terminal Disclaimer is attached hereto as EXHIBIT 12.

(c) No certificate of correction or reexamination certificate exists in respect of U.S. Patent 4,883,812.

(9) United States Patent Number 4,883,812 claims a method of using the active ingredient in the approved product FLOLAN® Injection. Applicant hereinbelow lists each applicable patent claim and demonstrates the manner in which each applicable patent claim reads on the method of using the approved product.

(a) Claim 3 reads as follows: "A method of treating a mammal for hypertension which comprises parenterally administering an effective antihypertensive amount of prostacyclin anion in a liquid to said mammal."

(1) The approved product, FLOLAN® (epoprostenol sodium) for Injection is an injectable solution containing a liquid and the salt, epoprostenol sodium.

(a) Epoprostenol sodium is prostacyclin sodium. See EXHIBIT 13.

(b) Since the salt of prostacyclin contains the prostacyclin anion (the prostacyclin molecule with a negatively charged carboxyl moiety) associated with a cation, *e.g.*, sodium, claim 3 reads on the approved product since it claims a method of using "prostacyclin anion" for treating hypertension.

(2) The approved product, FLOLAN® Injection, is indicated for the long-term intravenous treatment of primary pulmonary hypertension in NYHA Class III and Class IV patients.

(a) Primary pulmonary hypertension is a type of hypertension.

(b) 9.2 ng/kg/min is an effective antihypertensive amount of prostacyclin anion.

(c) The approved product is administered intravenously which is a type of parenteral administration.

(d) NYHA Class III and Class IV patients belong to the class *Mammalia.*

(b) Claim 4 reads as follows: "A method of claim 3 in which the prostacyclin anion is administered by injection."

(1) The approved product is administered intravenously which is accomplished by injection.

(10) The relevant dates and information pursuant to 35 U.S.C. 156(g) necessary to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

(a) Effective Date of IND
The Investigational New Drug ("IND") application for FLOLAN® Injection was filed May 30, 1979 and assigned IND number 16,459. The IND became effective 30 days thereafter on June 29, 1979.

(b) Issue Date of Patent
U.S. Patent No. 4,883,812 issued November 28, 1989 and claims a method of using new a drug.

(c) Submission Date of NDA
The NDA for FLOLAN® Injection was submitted February 28,1994 and assigned NDA number 20-444.

(d) Approval Date of NDA
NDA 20-444 for FLOLAN® Injection was approved by the FDA on September 20, 1995.

(11) A brief description of each significant activity undertaken by Applicant during both the IND and NDA regulatory periods is presented in chronological form and is attached hereto as EXHIBIT 7, "Due Diligence Log".

(a) The Due Diligence Log reflects significant communications between Applicant and FDA during regulatory periods. Such communications include, but are not limited to: submission of pre-clinical reports; registration of clinical protocols and amendments thereof; registration of clinical investigators and amendments thereof; submission of adverse event reports; submission of IND Annual Reports, etc.

(b) Periods between such communications enumerated in the Due Diligence Log reflect Applicant's diligent undertaking of the necessary clinical studies and other activities required by the FDA in order to obtain approval for Applicant's product.

(12) Applicant is of the opinion that U.S. Patent 4,883,812 is eligible for a 1346 day extension pursuant to 35 U.S.C. 156(g)(6)(A).

(a) Applicant has satisfied the eligibility criteria necessary to obtain a patent term extension pursuant to 35 U.S.C. 156.

(1) 35 U.S.C 156(a)
U.S. Patent 4,883,812 claims a method of using a drug product.

(2) 35 U.S.C. 156(a)(1)
The term of U.S. Patent 4,883,812 has not expired before submission of this application.

(3) 35 U.S.C. 156(a)(2)
The term of U.S. Patent 4,883,812 has never been extended.

(4) 35 U.S.C. 156(a)(3)
The application for extension is submitted by the owner of record in accordance with the requirements of 35 U.S.C. 156(d) and 37 C.F.R. 1.710 *et seq.*

(5) 35 U.S.C. 156(a)(4)
The approved product, FLOLAN® Injection, has been subject to a regulatory review period before its commercial marketing or use.

(6) 35 U.S.C. 156(a)(5)(A)
The commercial marketing or use of the approved product, FLOLAN® Injection, after the regulatory review period is the first permitted commercial marketing or use of the approved product under the provisions of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355) under which such regulatory review period occurred.

(b) Applicant herewith, claims a patent term extension of 1346 days for U.S. Patent 4,883,812 pursuant to 35 U.S.C. 156(g) as follows:

(1) One half of the IND regulatory review period for the approved product beginning November 28, 1989 (the IND period continuing from the date of the issuance of U.S. Patent 4,883,812) and ending on February 27, 1994 (one day prior to the date on which the NDA for the approved product was initially submitted), such sum being equal to 776 days.

(2) The full term of the NDA regulatory review period commencing February 28, 1994 (the date NDA 20-444 for the approved product was originally submitted) and ending on September 20, 1995 (the date on which NDA 20-444 was approved), such sum being equal to 570 days. See EXHIBIT 8.

(3) The sum of paragraphs (1) and (2) in this subsection equals 1346 days. Said sum of 1346 and does not exceed the maximum allowable extension of 5 years pursuant to 35 U.S.C. 156(g)(6)(A). See EXHIBIT 8.

(c) Applicant herewith, claims an expiration date of May 11, 2006 for U.S. Patent 4,883,812 pursuant to 35 U.S.C. 156(c)(3).

(1) The expiration of U.S. Patent 4,883,812, as terminally disclaimed against U.S. 4,539,333 is September 3, 2002.

(2) Extending the September 3, 2002 expiration by 1346 days would result in an expiration date of May 11, 2006.

(3) Being that expiration of U.S. Patent 4,883,812 believed to be entitled to said 1346 day extension is May 11, 2006, the limitation in 35 U.S.C. 156(c)(3) which requires that term extensions be reduced in order to limit the expiration date of a patent receiving term extensions to 14 years from the date of NDA approval is not reached, since 14 years from the date of NDA approval is September 20, 2009. See EXHIBIT 8.

(13) The Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to any determinations to be made relative to the application for extension. In this regard, Applicant wishes to disclose the following:

(a) In the late 1970's, an interference was declared by the USPTO between the application which led up to Moncada U.S. Patent 4,539,333 and one of the earlier applications which led up to the Upjohn (Johnson *et al.*) U.S. Patent 4,338,325.

(1) Both parties were contesting the priority rights related to the count which covered the inventions in both applications. However in the USPTO, it was decided that the claims present in the Moncada application to prostacyclin *per se* were to a product of nature and therefore were held unpatentable to Moncada and the interference was dissolved and patents issued to the respective parties.

(2) Upjohn, to the best of Applicant's knowledge, had a U.S. application in the interference with an earlier date as to the salts of epoprostenol, whereas the first UK priority application of the Moncada disclosure was limited to prostacyclin *per se.* Upjohn did not claim prostacyclin *per se* since that was not their invention.

(3) Subsequently, the Moncada application leading up to U.S. Patent 4,883,812 was filed for treating hypertension.

(b) With respect to the Johnson patent 4,338,325 cited in 4,883,812, there was no disclosure in Johnson '325 of the use of prostacyclin anion in the treatment of hypertension. Treatment of high blood pressure (hypertension) is based on the vasodilatory action of prostacyclin and its salts. (U.S. 4,883,812 Col.4, lines 24-30). At Col. 5, lines 33-35 of U.S. 4,883,812, there is disclosed liquid carriers.

(c) In addition to enclosing the above mentioned patents, Applicant attaches U.S. 4,335,139 which claims the formulation of the approved product. See EXHIBITS 9-11.

(14) The Commissioner of Patents and Trademarks is authorized to charge deposit account 02-4857 in the amount of $1,030.00 for receiving and acting upon this application for extension of term. In the event the actual fee differs from that specified above, it is requested that the overpayment be charged or the underpayment credited as authorized in the letter from David J. Levy, Ph.D. enclosed herewith.

(15) Inquiries and correspondence relating to this application for patent term extension are to be directed to:

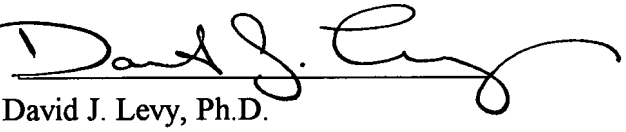
David J. Levy, Ph.D.
Patent Counsel, Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle Park, NC 27709
(919) 248-7656

(16) A duplicate of the application papers, certified as such is attached hereto.

(17) Submitted herewith is a Declaration by David J. Levy, Ph.D., Patent Counsel for Glaxo Wellcome Inc., which meets the criteria set forth in 37 C.F.R. 1.740(b).

The undersigned hereby certifies that this Application for Extension of Patent Term Under 35 U.S.C. 156 including its EXHIBITS and supporting papers is being submitted as duplicate originals.

Respectfully submitted,
Glaxo Wellcome Inc.

By: 
David J. Levy, Ph.D.
Patent Counsel

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re	:	U.S. Patent No. 4,883,812
Issued	:	November 28, 1989
Inventors	:	Salvador Moncada
Assignee	:	Burroughs Wellcome Co.
For	:	TREATMENT OF HYPERTENSION USING PROSTACYCLIN

Commissioner of Patent and Trademarks
Box Patent Extension
Washington, DC 20231

DECLARATION UNDER C.F.R. 1.740(b)

To the Commissioner of Patents and Trademarks:

I, David J. Levy, residing in Raleigh, North Carolina, declare as follows:

(1) That I am a patent attorney authorized to practice before the United States Patent and Trademark Office and that my registration number is 27,655.

(2) That I make this declaration as Patent Counsel for Glaxo Wellcome Inc., a corporation of the State of North Carolina and successor in interest to Burroughs Wellcome Co. by virtue of merger and name change, having a place of business at Five Moore Drive, Research Triangle Park, North Carolina 27709 and have general authority to act on its behalf in patent matters.

(3) That Burroughs Wellcome Co. was the assignee of the entire right, title and interest in United States Patent 4,883,812 issued November 28, 1989 (hereinafter "Patent") having now been succeeded in interest by Glaxo Wellcome Inc., by virtue of merger and name change.

(4) That I have reviewed and understand the contents of the APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. 156 submitted herewith on behalf of Glaxo Wellcome Inc. requesting a 1346 day extension of the term of the Patent.

(5) That said application is being submitted in the alternative with an application for extension of U.S. Patent 4,338,325, both in respect of FLOLAN® (epoprostenol sodium) for Injection, duly electing U.S. Patent 4,883,812 to be extended and in the alternative U.S. Patent 4,338,325 pursuant to 37 C.F.R. 1.785(b).

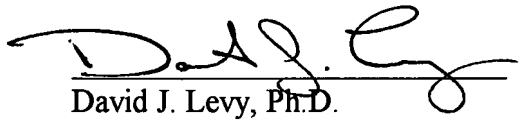
(6) That the aforementioned election is not intended to waive any right of appeal should said election or alternative be denied a term extension.

(7) That I believe that the Patent is subject to extension pursuant to 37 CFR 1.710.

(8) That I believe that a 1346 day extension of the term of the Patent is justified under 35 U.S.C. 156 and applicable regulations.

(9) That I believe the Patent meets the conditions for the extension of the term of a patent as set forth in 37 CFR 1.720.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of United States Patent 4,338,325 and any extensions thereof.



November 13, 1995

David J. Levy, Ph.D.
Reg. No. 27,655
Patent Counsel
Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle Park, NC 27709

Date

EXHIBIT 1

ASSIGNMENT OF U.S. PATENT 4,539,333
PARENT OF U.S. PATENT 4,883,812

4539333

A S S I G N M E N T

WHEREAS I, Salvador Moncada,

residing at 17 St. Davids Close, West Wickham. Kent. England.,

have invented or discovered certain improvements in "Ethers"

hereinafter referred to as said invention and improvements for which I have made a United States of America application for Letters Patent filed 10th May 1977 Serial No. 795 524, and;

WHEREAS BURROUGHS WELLCOME CO., a corporation organized and existing under and by virtue of the laws of the State of North Carolina and having its principal place of business at Research Triangle Park, North Carolina, is desirous of acquiring the whole right, title and interest in and to said invention and improvements and application and in and to any Letters Patent to be obtained therefor in the United States, its territories and possessions; and

WHEREAS THE WELLCOME FOUNDATION LTD, a company incorporated in England, whose registered office is at 183-193 Euston Road, London, N.W.1, England, is desirous of acquiring the whole right, title and interest in and to said invention and improvements and in and to any applications for said invention and improvements and any Letters Patent to be obtained therefor in all countries other than the United States, its territories and possessions;

NOW, THEREFORE, to all whom it may concern, be it known that I, Salvador Moncada

for good and valuable considerations unto me moving, the receipt whereof is hereby acknowledged, have sold, assigned and transferred, and by these presents do sell, assign and transfer my whole right,

title and interest in and to said invention and improvements to said BURROUGHS WELLCOME CO., throughout the United States of America, its territories and possessions, and in and to said application and any renewals, continuations, continuations-in-part, and any divisions thereof, and in and to any and all Letters Patent of the United States of America which may be granted on said invention and improvements and any reissue thereof including any priority rights under the International convention for the United States of America;

AND, MY whole right, title and interest in and to said invention and improvements to THE WELLCOME FOUNDATION LTD, in all other countries throughout the world, and in and to any applications in said other countries, and continuations-in-part, patents of addition, revalidation patents, patents of importation, registrations, and any renewals, extensions and divisions thereof, and in and to any and all Letters Patent of said all other countries which may be granted on said invention and improvements including any priority rights under the International Convention for all other countries of the Union;

AND, I do hereby authorize and request the issue of any Letters Patent in the respective areas referred to, to said BURROUGHS WELLCOME CO. or THE WELLCOME FOUNDATION LTD, as assignees of my whole right, title and interest in and to the same for the sole use and behoof of the said assignees, their successors and assigns as their interests appear herein;

AND, I warrant that I have not knowingly conveyed to others any right in said invention, improvements, applications or patents or any license to use the same or to make, use or sell anything embodying or utilizing said invention and improvements and that I have good right to assign the same to BURROUGHS WELLCOME CO. or THE WELLCOME FOUNDATION LTD;

AND, I the undersigned Salvador Moncada for the consideration aforesaid, do hereby agree that I or my executors or legal representatives, will provide information and make, execute and deliver any and all other instruments in writing, and any and all further acts, application papers, affidavits, assignments and other documents which may be necessary or desirable to more effectually secure to and vest in said BURROUGHS WELLCOME CO. and THE WELLCOME FOUNDATION LTD, their successors and assigns, the whole right, title and interest in and to the said invention and improvements, applications, Letters Patent, rights, title and interest, hereby sold, assigned and conveyed, or intended so to be.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed my seal this eighth day of November 1977,

Inventor's Signature Salvador Moncada
SALVADOR MONCADA

GREAT BRITAIN)
ENGLAND) ss.
COUNTY OF KENT)

REEL 3479 FRAME 685

On this eighth day of November 1977, before me personally came SALVADOR MONCADA to me known and known to me to be the individual described in and who executed the foregoing assignment, and he thereupon acknowledged to me that he executed same.

DE FINNA, SCORERS & JOHN YENN
161,
SALISBURY HOUSE
LONDON WALL
LONDON, E.C.2 M.

Notary Public of London, England

GREAT BRITAIN AND NORTHERN IRELAND)
LONDON, ENGLAND) ss.
EMBASSY OF THE UNITED STATES OF AMERICA)

I, Leslie A. Gerson Vice Consul of the United States of America residing at London, England, duly commissioned and qualified, do hereby certify that

EDWIN BRUCE WALKER

whose signature and official seal are respectively subscribed and affixed to the annexed certificate, was on the date of the signing thereof a Notary Public at London, England, duly authorized to perform notarial acts, duly appointed and qualified, to whose official acts faith and credit are due; that I have compared the signature of said

EDWIN BRUCE WALKER REEL 3479 FRAME 686

on the annexed certificate with a specimen of his signature filed in this Embassy; that I believe his signature to be genuine; that I have compared the impression of the seal affixed thereto with a specimen thereof filed in this Embassy, and that I believe the impression of the seal upon the said original annexed certificate to be genuine.

IN WITNESS WHEREOF I have hereunto set my hand and affixed the seal of the Consular Service of the United States of America at London, England, this tenth day of November in the year of Our Lord one thousand nine hundred and seventy-seven.

Leslie Ann Gerson
Leslie A. Gerson
Vice Consul of the United States of America at London, England.

Service Receipt No. G1477331

Tariff Item No. 49

Fee: ~~$2.50~~ $3.00

~~No fee prescribed~~

RECORDED
U.S. PATENT OFFICE
DEC - 7 1977

Lutrelle F. Parker
ACTING COMMISSIONER OF
PATENTS & TRADEMARKS

LND/129
Feb. 77

EXHIBIT 2

CERTIFICATE OF MERGER
&
CERTIFICATE OF CHANGE OF NAME

STATE OF NORTH CAROLINA

Department of The Secretary of State

To all whom these presents shall come, Greetings:

I, **Rufus L. Edmisten,** *Secretary of State of the State of North Carolina, do hereby certify the following and hereto attached to be a true copy of*

ARTICLES OF MERGER

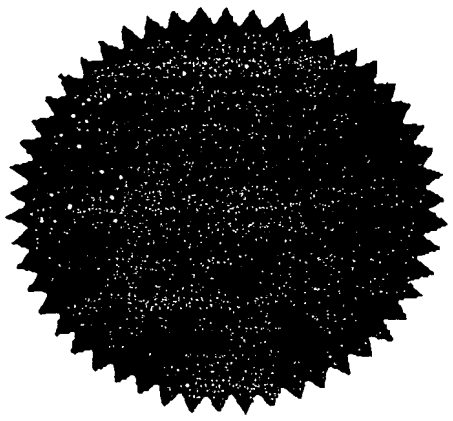
OF
GLAXO WELLCOME INC.
a North Carolina Corporation

INTO

BURROUGHS WELLCOME CO.
a North Carolina Corporation

the original of which was filed in this office on the 30th day of October, 1995.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed my official seal at the City of Raleigh, this 30th day of October, 1995.



Rufus L. Edmisten

Secretary of State

STATE OF NORTH CAROLINA

Department of The Secretary of State

To all whom these presents shall come, Greetings:

I, Rufus L. Edmisten, *Secretary of State of the State of North Carolina, do hereby certify the following and hereto attached to be a true copy of*

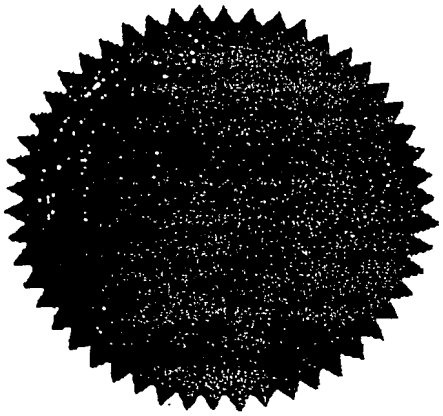
ARTICLES OF AMENDMENT

OF

BURROUGHS WELLCOME CO.
name changed to:
GLAXO WELLCOME INC.

the original of which was filed in this office on the 30th day of October, 1995.

IN WITNESS WHEREOF, I have hereunto set my hand and affixed my official seal at the City of Raleigh, this 30th day of October, 1995.



Rufus L. Edmisten

Secretary of State

EXHIBIT 3

MAINTENANCE FEE STATEMENT for U.S. PATENT 4,883,812

NO INFORMATION AVAILABLE IN THE AUTOMATED RECORDS
FOR PATENT NUMBER 4883812

AUTOMATED DATA COVERS ASSIGNMENTS RECORDED FROM AUGUST 5, 1980 TO DATE
FOR ASSIGNMENTS RECORDED BEFORE AUGUST 5, 1980, SEE CARD INDEX.

TO PROCESS ANOTHER QUERY PRESS THE 'P' AND 'XMIT' KEYS.

IF NO ADDITIONAL PATENT NUMBER QUERIES ARE DESIRED, PRESS 'XMIT'.

ENTER RESPONSE 10/23/95 13:07

Patent Maintenance Fees - Public Inquiry

Patent#: 4883812 Filed: 08/29/88 Issued: 11/28/89 Serial#: 07237987
Status: 8th Year Fee Window Opens: 11/28/96 Sml Entity: NO
Window Opens: 11/29/96 Surchg Due: 05/28/97 Expiration: 11/28/97
Fee Amt Due:$ 1990 Surchg Amt Due:$ Total Amt Due:$ 1990
Fee Code: 184 Surchg Code:
Title: TREATMENT OF HYPERTENSION USING PROSTACYCLIN

Address For Fee Purposes:
OLCOTT INTERNATIONAL & CO.
62 HACKENSACK PLANK ROAD
P.O. 3014
WEEHAWKEN, NJ. 07087

Most Recent Significant Events:
05/14/93 Payment of Maintenance Fee, 4th Year, Large Entity
09/28/90 Payor Number Assigned
Last Event On Maintenance History

EXHIBIT 4

FDA APPROVAL LETTER FOR
FLOLAN® (epoprostenol sodium) for Injection

#12

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re	:	U.S. Patent No. 4,883,812
Issued	:	November 28, 1989
Inventors	:	Salvador Moncada
Assignee	:	Burroughs Wellcome Co.
For	:	TREATMENT OF HYPERTENSION USING PROSTACYCLIN

Re: Patent Term Extension for U.S. Patent 4,338,325

Commissioner of Patents and Trademarks
Box Patent Extension
Washington, DC 20231

Sir:

Transmitted herewith is an APPLICATION FOR EXTENSION OF PATENT TERM under 35 U.S.C. 156 with regard to U.S. Patent No. 4,883,812.

The Commissioner is hereby authorized to charge Deposit Account No. 02-4857 in the amount of $1,030.00 to cover the application fee. The Commissioner is hereby authorized to charge any additional fees, which may be required, or credit overpayment to Account No. 02-4857. Triplicate copies of this letter are enclosed.

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this Application for Patent term Extension and the documents referred to therein are being deposited with the United States Postal Service on this date _____, 1995 in an envelope as "Express Mail Post Office to Addressee," Mailing Label Number _____ addressed to the: Commissioner of Patents and Trademarks Washington, D.C. 20231.

_____
Type or print name of person mailing paper)

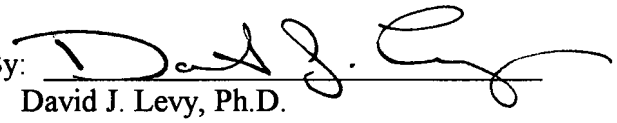
_____
(Signature of person mailing paper)

Please address all communications relating to the enclosed APPLICATION FOR EXTENSION OF PATENT TERM to:

David J. Levy, Ph.D.
Patent Counsel
Glaxo Wellcome Inc.
Intellectual Property Department
Five Moore Drive
Research Triangle Park, NC 27709

Telephone No. (919) 248-7656

Respectfully submitted,
Glaxo Wellcome Inc.

By: 
David J. Levy, Ph.D.
Reg. No. 27,655
Patent Counsel

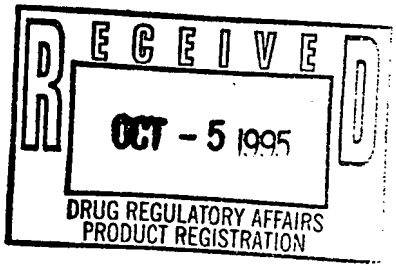
DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

SEP 20 1995

NDA 20-444



Burroughs Wellcome Company
Attention: Michael J. Dalton, Pharm.D.
3030 Cornwallis Road
Research Triangle Park, North Carolina 27709

Dear Dr. Dalton:

Please refer to your February 28, 1994 new drug application submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Flolan (epoprostenol sodium) for Injection.

We acknowledge receipt of your amendments dated April 28, May 22, June 6 and 9, and July 24, 1995.

This new drug application provides for the long-term intravenous treatment of primary pulmonary hypertension in NYHA Class III and Class IV adult patients.

We have completed the review of this application including the submitted draft labeling and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed marked-up draft labeling. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed marked-up draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

Please submit sixteen copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-444. Approval of this labeling by FDA is not required before it is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

We remind you of your Phase 4 commitments that, along with completion dates, are listed below:

1. The commitments agreed to in the April 27, 1995 telephone conversation between representatives of your firm and this Agency, and detailed in an Agency letter dated April 28, 1995 to submit to FDA additional information concerning the sterilization process.

2. The commitments agreed to in your April 10, 1995 submission, and confirmed in an Agency letter dated April 28, 1995, to submit to FDA, within 6 months of approval of the NDA, additional information regarding chemistry, manufacturing, and controls.

Protocols, data, and final reports should be submitted to your IND for this product and a copy of the cover letter sent to this NDA. Should an IND not be required to meet your Phase 4 commitments, please submit protocol, data, and final reports to this NDA as correspondences. For administrative purposes, all submissions, including labeling supplements, relating to these Phase 4 commitments must be clearly designated "Phase 4 Commitments."

In addition, please submit three copies of the introductory promotional material that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Please submit one copy to the Division of Gastrointestinal and Coagulation Drug Products and two copies of both the promotional material and the package insert directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising and
Communications, HFD-240
5600 Fishers Lane
Rockville, Maryland 20857

Validation of the regulatory methods has not been completed. At the present time, it is the policy of the Center not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any problems that may be identified.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact:

Karen Oliver
Consumer Safety Officer
(301) 443-0487

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Evaluation 1
Center for Drug Evaluation and Research

ENCLOSURE

EXHIBIT 5

APPROVED PACKAGE INSERT FOR
FLOLAN® (epoprostenol sodium) for Injection
NDA 20-444

PACKAGE INSERT

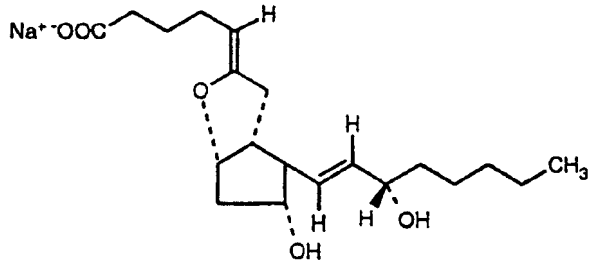
FLOLAN® (epoprostenol sodium) for Injection

DESCRIPTION: FLOLAN (epoprostenol sodium) for Injection is a sterile sodium salt formulated for intravenous administration. Each vial of FLOLAN contains epoprostenol sodium equivalent to either 0.5 mg (500,000 ng) or 1.5 mg (1,500,000 ng) epoprostenol, 3.76 mg glycine, 2.93 mg sodium chloride, and 50 mg mannitol. Sodium hydroxide may have been added to adjust pH.

Epoprostenol (PGI_2, PGX, prostacyclin), a metabolite of arachidonic acid, is a naturally occurring prostaglandin with potent vasodilatory activity and inhibitory activity of platelet aggregation.

Epoprostenol is (5Z,9 ,11 ,13*E*,15*S*)-6,9-epoxy-11,15-dihydroxyprosta-5,13-dien-1-oic acid.

Epoprostenol sodium has a molecular weight of 374.45 and a molecular formula of $C_{20}H_{31}NaO_5$. The structural formula is:



FLOLAN is a white to off-white powder that must be reconstituted with STERILE DILUENT for FLOLAN. STERILE DILUENT for FLOLAN is supplied in 50 mL glass vials containing 94 mg glycine, 73.5 mg sodium chloride, sodium hydroxide (added to adjust pH), and Water for Injection, USP.

The reconstituted solution of FLOLAN has a pH of 10.2 to 10.8 and is increasingly unstable at a lower pH.

CLINICAL PHARMACOLOGY:

General: Epoprostenol has two major pharmacological actions: (1) direct vasodilation of pulmonary and systemic arterial vascular beds, and (2) inhibition of platelet aggregation. In animals, the vasodilatory effects reduce right and left ventricular afterload and increase cardiac output and stroke volume. The effect of epoprostenol on heart rate in animals varies with dose. At low doses, there is

vagally mediated bradycardia, but at higher doses, epoprostenol causes reflex tachycardia in response to direct vasodilation and hypotension. No major effects on cardiac conduction have been observed. Additional pharmacologic effects of epoprostenol in animals include bronchodilation, inhibition of gastric acid secretion, and decreased gastric emptying.

Pharmacokinetics: Epoprostenol is rapidly hydrolyzed at neutral pH in blood and is also subject to enzymatic degradation. Animal studies using tritium-labelled epoprostenol have indicated a high clearance (93 mL/min/kg), small volume of distribution (357 mL/kg), and a short half-life (2.7 minutes). During infusions in animals, steady-state plasma concentrations of tritium-labelled epoprostenol were reached within 15 minutes and were proportional to infusion rates.

No available chemical assay is sufficiently sensitive and specific to assess the in vivo human pharmacokinetics of epoprostenol. The in vitro half-life of epoprostenol in human blood at 37°C and pH 7.4 is approximately 6 minutes; the in vivo half-life of epoprostenol in man is therefore expected to be no greater than 6 minutes. The in vitro pharmacologic

half-life of epoprostenol in human plasma, based on inhibition of platelet aggregation, was similar for males (n = 954) and females (n = 1024).

Tritium-labelled epoprostenol has been administered to humans in order to identify the metabolic products of epoprostenol. Epoprostenol is metabolized to two primary metabolites: 6-keto-$PGF_{1\alpha}$ (formed by spontaneous degradation) and 6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$ (enzymatically formed), both of which have pharmacological activity orders of magnitude less than epoprostenol in animal test systems. The recovery of radioactivity in urine and feces over a one-week period was 82% and 4% of the administered dose, respectively. Fourteen additional minor metabolites have been isolated from urine, indicating that epoprostenol is extensively metabolized in man.

Clinical Trials:

DELETE and REPLACE with:
CLINICAL TRIALS: IN PRIMARY PULMONARY HYPERTENSION (PPH

Hemodynamic Effects: Acute intravenous infusions of FLOLAN for up to 15 minutes in patients with secondary and primary pulmonary hypertension (PPH) produce dose-related increases in cardiac index (CI) and stroke volume (SV), and dose-related

CORRECT TYPOGRAPHICAL ERROR:
(Insert space between run-on words).

decreases in pulmonary vascular resistance (PVR), total pulmonary resistance (TPR), and mean systemic arterial pressure (SAPm). The effects of FLOLAN on mean pulmonary artery pressure (PAPm) in patients with PPH were variable and minor.

Chronic continuous infusions of FLOLAN in patients with PPH were studied in two prospective, open, randomized trials of 8 and 12 weeks duration comparing FLOLAN plus standard therapy to standard therapy alone. Dosage of FLOLAN was determined as described in DOSAGE AND ADMINISTRATION and averaged 9.2 ng/kg/min ~~at the end of the 12-week trial.~~ Standard therapy varied among patients and included some or all of the following: anticoagulants in essentially all patients; oral vasodilators, diuretics, and digoxin in one-half to two-thirds of patients; and supplemental oxygen in about half the patients. Except for two New York Heart Association (NYHA) functional Class II patients, all patients were either functional Class III or Class IV. As results were similar in the two studies, the pooled results are described. Chronic hemodynamic effects were generally similar to acute effects. CI, SV, and arterial oxygen saturation were increased, and PAPm, right atrial pressure (RAP), TPR, and systemic vascular resistance (SVR) were decreased in patients who received FLOLAN chronically compared to those who did not. Table 1 illustrates the treatment-related hemodynamic changes in these patients after 8 or 12 weeks of treatment.

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Table 1

Hemodynamics During Chronic Administration of FLOLAN

Hemodynamic Parameter	Baseline		Mean change from baseline at end of treatment period*	
	FLOLAN (n = 52)	Standard Therapy (n = 54)	FLOLAN (n = 48)	Standard Therapy (n = 41)
CI (L/min/m^2)	2.0	2.0	0.3"	-0.1
PAPm (mm Hg)	60	60	-5"	1
PVR (Wood U)	16	17	-4"	1
SAPm (mm Hg)	89	91	-4	-3
SV (mL/beat)	44	43	6"	-1
TPR (Wood U)	20	21	-5"	1

* At 8 weeks: FLOLAN n = 10; Standard Therapy n = 11.
At 12 weeks: FLOLAN n = 38; Standard Therapy n = 30.
"Denotes statistically significant change between FLOLAN and Standard Therapy groups.

CI = cardiac index; PAPm=mean pulmonary arterial pressure; PVR = pulmonary vascular resistance; SAPm = mean systemic arterial pressure; SV = stroke volume; TPR = total pulmonary resistance.

These hemodynamic improvements appeared to persist when FLOLAN was administered for at least 36 months in an open, non-randomized study.

Clinical Effects: Exercise capacity, as measured by the 6-minute walk test, improved significantly in patients receiving continuous intravenous FLOLAN plus standard therapy for 8 or 12 weeks compared to those receiving standard therapy alone. Improvements were apparent as early as the first week of therapy~~, and in long-term follow-up appeared to be maintained beyond 2 years.~~ Increases in

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exercise capacity were accompanied by significant improvement in dyspnea and fatigue, as measured by the Congestive Heart Failure Questionnaire and the Dyspnea Fatigue Index.

Survival was improved in NYHA functional Class III and Class IV PPH patients treated with FLOLAN for 12 weeks in a multicenter, open, randomized, parallel study. At the end of the treatment period, 8 of 40 patients receiving standard therapy alone died, whereas none of the 41 patients receiving FLOLAN died ($P = 0.003$).

INDICATIONS AND USAGE: FLOLAN is indicated ~~INSERT: in adults~~ for the long-term intravenous treatment of primary pulmonary hypertension in NYHA Class III and Class IV patients (see CLINICAL PHARMACOLOGY: Clinical Trials).

CONTRAINDICATIONS: A large study evaluating the effect of FLOLAN on survival in NYHA Class III and IV patients with CHF due to severe left ventricular systolic dysfunction was terminated after an interim analysis of 471 patients revealed a higher mortality in patients receiving FLOLAN plus standard therapy than in those receiving standard therapy alone. The

chronic use of FLOLAN in patients with CHF due to severe left ventricular systolic dysfunction is therefore contraindicated

FLOLAN is also contraindicated in patients with known hypersensitivity to the drug or to structurally-related compounds.

WARNINGS: FLOLAN must be reconstituted only as directed using STERILE DILUENT for FLOLAN. FLOLAN must not be reconstituted or mixed with any other parenteral medications or solutions prior to or during administration.

Abrupt Withdrawal: Abrupt withdrawal (including interruptions in drug delivery) or sudden large reductions in dosage of FLOLAN may result in symptoms associated with rebound pulmonary hypertension, including dyspnea, dizziness, and asthenia. In clinical trials, one Class III PPH patient's death was judged attributable to the interruption of FLOLAN. Abrupt withdrawal should be avoided.

Pulmonary Edema: Some patients with primary pulmonary hypertension have developed pulmonary edema during dose ranging, which may be

associated with pulmonary veno-occlusive disease. FLOLAN should not be used chronically in patients who develop pulmonary edema during dose ranging.

Sepsis: See ADVERSE REACTIONS: Adverse Events Attributable to the Drug Delivery System.

PRECAUTIONS:

General: FLOLAN should be used only by clinicians experienced in the diagnosis and treatment of PPH. The diagnosis of PPH should be carefully established by standard clinical tests to exclude secondary causes of pulmonary hypertension.

FLOLAN is a potent pulmonary and systemic vasodilator. Dose ranging with FLOLAN must be performed in a setting with adequate personnel and equipment for physiologic monitoring and emergency care. Although dose ranging in clinical trials was performed during right heart catheterization employing a pulmonary artery catheter, the risk of cardiac catheterization in patients with PPH should be carefully weighed against the potential benefits. During acute dose ranging, asymptomatic increases in pulmonary artery pressure coincident with increases in cardiac output occurred rarely. In such cases, dose reduction should be considered, but

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Although dose ranging in clinical trials was performed during right heart catheterization employing a pulmonary artery catheter, in uncontrolled studies utilizing Flolan, the risk of cardiac catheterization in patients with PPH should be carefully weighed against the potential benefits.

such an increase does not imply that chronic treatment is contraindicated.

During chronic use, FLOLAN is delivered continuously on an ambulatory basis through a permanent indwelling central venous catheter. Unless contraindicated, anticoagulant therapy should be administered to PPH patients receiving FLOLAN to reduce the risk of pulmonary thromboembolism or systemic embolism through a patent foramen ovale. In order to reduce the risk of infection, aseptic technique must be used in the reconstitution and administration of FLOLAN as well as in routine catheter care. Because FLOLAN is metabolized rapidly, even brief interruptions in the delivery of FLOLAN may result in symptoms associated with rebound pulmonary hypertension including dyspnea, dizziness, and asthenia. The decision to initiate therapy with FLOLAN should be based upon the understanding that there is a high likelihood that intravenous therapy with FLOLAN will be needed for prolonged periods, possibly years, and the patient's ability to accept and care for a permanent intravenous catheter and infusion pump should be carefully considered.

Based on clinical trials, the acute hemodynamic

response to FLOLAN did not correlate well with improvement in exercise tolerance or survival during chronic use of FLOLAN. Dosage of FLOLAN during chronic use should be adjusted at the first sign of recurrence or worsening of symptoms attributable to PPH or the occurrence of adverse events associated with FLOLAN (see DOSAGE AND ADMINISTRATION). Following dosage adjustments, standing and supine blood pressure and heart rate should be monitored closely for several hours.

Information for Patients: Patients receiving FLOLAN should receive the following information:

FLOLAN must be reconstituted only with STERILE DILUENT for FLOLAN. FLOLAN is infused continuously through a permanent indwelling central venous catheter via a small, portable infusion pump. Thus, therapy with FLOLAN requires commitment by the patient to drug reconstitution, drug administration, and care of the permanent central venous catheter. Sterile technique must be adhered to in preparing the drug and in the care of the catheter, and even brief interruptions in the delivery of FLOLAN may result in rapid symptomatic deterioration. The decision to receive FLOLAN for PPH should be based upon the understanding that there is a high likelihood that

therapy with FLOLAN will be needed for prolonged periods, possibly years, and the patient's ability to accept and care for a permanent intravenous catheter and infusion pump should be carefully considered.

Drug Interactions: Additional reductions in blood pressure may occur when FLOLAN is administered with diuretics, antihypertensive agents, or other vasodilators. When other anti-platelet agents or anticoagulants are used concomitantly, there is the potential for FLOLAN to increase the risk of bleeding. However, patients receiving FLOLAN infusions in clinical trials were maintained on anticoagulants without evidence of increased bleeding. In clinical trials, FLOLAN was used with digoxin, diuretics, anticoagulants, oral vasodilators, and supplemental oxygen.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term studies in animals have not been performed to evaluate carcinogenic potential. A micronucleus test in rats revealed no evidence of mutagenicity. The Ames test and DNA elution tests were also negative, although the instability of epoprostenol makes the significance of these tests uncertain. Fertility was not impaired in rats given

FLOLAN by subcutaneous injection at doses up to 100 µg/kg/day, [600 g/m²/day, 2.5 times the recommended human dose (4.6 ng/kg/min or 245.1 g/m²/day, i.v.) based on body surface area].

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Pregnancy: Pregnancy Category B. Reproductive studies have been performed in pregnant rats and rabbits at doses up to 100 g/kg/day (600 g/m²/day in rats, 2.5 times the recommended human dose, and 1180 g/m²/day in rabbits, 4.8 times the recommended human dose based on body surface area) and have revealed no evidence of impaired fertility or harm to the fetus due to FLOLAN. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

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Labor and Delivery: The use of FLOLAN during labor, vaginal delivery, or caesarean section has not been adequately studied in humans.

Nursing Mothers: It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when FLOLAN is administered to a nursing woman.

Pediatric Use: No adequate and well-controlled studies have been performed in pediatric patients. However, sixty-three pediatric patients less than 16 years of age with pulmonary hypertension have received FLOLAN during acute dose ranging. The mean maximum dose in patients less than 16 years of age was significantly greater than the mean maximum dose in adults during acute dose ranging (25 ng/kg/min and 8.6 ng/kg/min, respectively). A limited number of pediatric patients less than 16 years of age (n=5) with PPH in NYHA functional Class III or Class IV have received FLOLAN chronically in controlled clinical trials. In contrast to acute dose ranging, mean chronic infusion rates were only slightly higher for pediatric patients than for adults; this difference did not reach statistical significance. There were no important differences in the adverse event profiles between adult and pediatric patients.

DELETE and replace with

Pediatric Use: Safety and effectiveness in pediatric patients have not been established.

Geriatric Use: Clinical studies of FLOLAN did not include sufficient numbers of patients aged 65 and over to determine whether they respond differently from younger patients. In general, dose selection for an elderly patient should be cautious, reflecting the greater frequency of decreased hepatic, renal, or

cardiac function and of concomitant disease or other drug therapy.

ADVERSE REACTIONS: During clinical trials, adverse events were classified as follows: (1) adverse events during acute dose ranging, (2) adverse events during chronic dosing, and (3) adverse events associated with the drug delivery system.

Adverse Events During Acute Dose Ranging: During acute dose ranging, FLOLAN was administered in 2 ng/kg/min increments until the patients developed symptomatic intolerance. The most common adverse events and the adverse events that limited further increases in dose were generally related to the major pharmacologic effect of FLOLAN, vasodilation. The most common dose-limiting adverse events were nausea and vomiting, headache, hypotension, chest pain, dizziness, and bradycardia. Table 2 lists the adverse events reported during acute dose ranging in decreasing order of frequency.

Table 2

Adverse Events During Acute Dose Ranging

Adverse Events Occurring in ≥1% of Patients	FLOLAN (% of patients) (n = 391)
Flushing	58
Headache	49
Nausea/Vomiting	32
Hypotension	16
Anxiety, nervousness, agitation	11
Chest pain	11
Dizziness	8
Bradycardia	5
Abdominal pain	5
Musculoskeletal pain	3
Dyspnea	2
Back pain	2
Sweating	1
Dyspepsia	1
Hypesthesia/Paresthesia	1
Tachycardia	1

Adverse Events During Chronic Administration:

Interpretation of adverse events is complicated by the clinical features of PPH, which are similar to some of the pharmacologic effects of FLOLAN (e.g., dizziness, syncope). Adverse events probably related to the underlying disease include dyspnea, fatigue, chest pain, right ventricular failure, and pallor. Several adverse events, on the other hand, can clearly be attributed to FLOLAN. These include headache, jaw pain, flushing, diarrhea, nausea and vomiting, flu-like symptoms, and anxiety/nervousness. In an effort to separate the adverse effects of the drug from the adverse effects of the underlying

disease, table 3 lists adverse events that occurred at a rate at least 10% different in the two groups in controlled trials.

Table 3

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Adverse Events Regardless of Attribution Occurring with 10% Difference Between FLOLAN and Standard Therapy Alone

Adverse Event	FLOLAN (% of patients) (n = 52)	Standard therapy (% of patients) (n = 54)
Occurrence More Common with FLOLAN		
GENERAL		
Chills/Fever/Sepsis/Flu-like symptoms	25	11
CARDIOVASCULAR		
Tachycardia	35	24
Flushing	42	2
GASTROINTESTINAL		
Diarrhea	37	6
Nausea/Vomiting	67	48
MUSCULOSKELETAL		
Jaw Pain	54	0
Myalgia	44	31
Non-specific musculoskeletal pain	35	15
NEUROLOGICAL		
Anxiety/nervousness/tremor	21	9
Dizziness	83	70
Headache	83	33
Hypesthesia, Hyperesthesia, Paresthesia	12	2
Occurrence More Common With Standard Therapy		
CARDIOVASCULAR		
Heart failure	31	52
Syncope	13	24
Shock	0	13
RESPIRATORY		
Hypoxia	25	37

Thrombocytopenia has been reported during uncontrolled clinical trials in patients receiving FLOLAN.

Table 4 lists additional adverse events reported in PPH patients receiving FLOLAN plus standard

436 therapy or standard therapy alone during controlled clinical trials.

Table 4

Adverse Events Regardless of Attribution Occurring with <10% Difference Between FLOLAN and Standard Therapy Alone

GENERAL		
Asthenia	87	81
CARDIOVASCULAR		
Angina Pectoris	19	20
Arrhythmia	27	20
Bradycardia	15	9
Supraventricular tachycardia	8	0
Pallor	21	30
Cyanosis	31	39
Palpitation	63	61
Cerebrovascular accident	4	0
Hemorrhage	19	11
Hypotension	27	31
Myocardial ischemia	2	6
GASTROINTESTINAL		
Abdominal pain	27	31
Anorexia	25	30
Ascites	12	17
Constipation	6	2
METABOLIC		
Edema	60	63
Hypokalemia	6	4
Weight reduction	27	24
Weight gain	6	4
MUSCULOSKELETAL		
Arthralgia	6	0
Bone pain	0	4
Chest pain	67	65
NEUROLOGICAL		
Confusion	6	11
Convulsion	4	0
Depression	37	44
Insomnia	4	4
RESPIRATORY		
Cough increase	38	46
Dyspnea	90	85
Epistaxis	4	2
Pleural effusion	4	2
DERMATOLOGIC		
Pruritus	4	0
Rash	10	13

Sweating	15	20
SPECIAL SENSES		
Amblyopia	8	4
Vision abnormality	4	0

Adverse Events Attributable to the Drug Delivery System: Chronic infusions of FLOLAN are delivered using a small, portable infusion pump through an indwelling central venous catheter. During controlled trials of up to 12 weeks duration, 21% of patients reported a local infection and 13% of patients reported pain at the injection site. During long-term follow-up, sepsis was reported at least once in 14% of patients and occurred at a rate of 0.32 infections per patient per year in patients treated with FLOLAN. This rate was higher than reported in patients using chronic indwelling central venous catheters to administer parenteral nutrition, but lower than reported in oncology patients using these catheters. Malfunctions in the delivery system resulting in an inadvertent bolus of or a reduction in FLOLAN were associated with symptoms related to excess or insufficient FLOLAN, respectively (see ADVERSE REACTIONS: Adverse Events During Chronic Administration and OVERDOSAGE).

OVERDOSAGE: Signs and symptoms of excessive doses of FLOLAN during clinical trials are the expected dose-limiting pharmacologic effects of

If the words "and OVERDOSAGE" are to be retained, the events described in the second paragraph of the OVERDOSAGE section should include its relationship to the delivery system.

FLOLAN, including flushing, headache, hypotension, tachycardia, nausea, vomiting, and diarrhea. Treatment will ordinarily require dose reduction of FLOLAN.

One patient with secondary pulmonary hypertension accidentally received 50 mL of an unspecified concentration of FLOLAN. The patient vomited and became unconscious with an initially unrecordable blood pressure. FLOLAN was discontinued and the patient regained consciousness within seconds. No fatal events have been reported following overdosage of FLOLAN.

Single intravenous doses of FLOLAN at 10 and 50 mg/kg, 2.5 million times a human dose of 20 g/kg for 15 minutes on a g/m² basis, were lethal to mice and rats, respectively. Symptoms of acute toxicity were hypoactivity, ataxia, loss of righting reflex, deep slow breathing, and hypothermia.

DELETE and REPLACE with:
Single intravenous doses of FLOLAN at 10 and 50 mg/kg (2703 and 27027 times the recommended acute phase human dose based on body surface area) were lethal to mice and rats, respectively. Symptoms of acute toxicity were hypoactivity, ataxia, loss of righting reflex, deep slow breathing, and hypothermia.

DOSAGE AND ADMINISTRATION:

Important Note: FLOLAN must be reconstituted only with STERILE DILUENT for FLOLAN. Reconstituted solutions of FLOLAN must not be diluted or administered with other parenteral solutions or medications (see WARNINGS).

Dosage:

Acute Dose Ranging:

The initial chronic infusion rate of FLOLAN is determined by an acute dose-ranging procedure. During controlled clinical trials, this procedure was performed during cardiac catheterization (see PRECAUTIONS), but in subsequent uncontrolled clinical trials, acute doseranging was performed without cardiac catheterization. In either case, the infusion rate is initiated at 2 ng/kg/min and increased in increments of 2 ng/kg/min every 15 minutes or longer until dose-limiting pharmacologic effects are elicited. The most common dose-limiting pharmacologic effects during dose ranging are nausea, vomiting, headache, hypotension, ~~chest pain~~, ~~dizziness, and bradycardia~~. During acute dose ranging in clinical trials, the mean maximum dose which did not elicit dose-limiting pharmacologic

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effects was 8.6 ± 0.3 ng/kg/min.

Continuous Chronic Infusion:

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Adults:

Chronic continuous infusion of FLOLAN should be administered through a central venous catheter. Temporary peripheral intravenous infusions may be used until central access is established. Chronic infusions of FLOLAN should be initiated at 4 ng/kg/min less than the maximum-tolerated infusion rate determined during acute dose ranging. If the maximum-tolerated infusion rate is less than 5 ng/kg/min, the chronic infusion should be started at one-half the maximum-tolerated infusion rate. During clinical trials, the mean initial chronic infusion rate was 5 ng/kg/min.

Dosage Adjustments: Changes in the chronic infusion rate should be based on persistence, recurrence, or worsening of the patient's symptoms of PPH and the occurrence of adverse events due to excessive doses of FLOLAN. In general, increases in dose from the initial chronic dose should be expected. In the controlled 12-week trial, for example, the dose increased from a mean starting dose of 5.2 g/kg/min (4 g/kg/min less than the new tolerated dose) to 9.2 g/kg/min by the end of week 12, just 1.6 g/kg/min less than the mean non-tolerated dose.

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Increments in dose should be considered if symptoms of PPH persist or recur after improving. The infusion should be increased by 1 to 2 ng/kg/min increments at intervals sufficient to allow assessment of clinical response; these intervals should be at least 15 minutes. Following establishment of a new chronic infusion rate, the patient should be observed

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and standing and supine blood pressure and heart rate monitored for several hours to ensure that the new dose is tolerated.

During chronic infusion, the occurrence of dose-related pharmacological events similar to those

observed during acute dose ranging may necessitate a decrease in infusion rate, but the adverse event may occasionally resolve without dosage adjustment. Dosage decreases should be made gradually in 2 ng/kg/min decrements every 15 minutes or longer until the dose-limiting effects resolve. Abrupt withdrawal of FLOLAN or sudden large reductions in infusion rates should be avoided. Except in life-threatening situations (e.g., unconsciousness, collapse, etc.), infusion rates of FLOLAN should be adjusted only under the direction of a physician.

In patients receiving lung transplants, doses of FLOLAN were tapered after the initiation of cardiopulmonary bypass.

Administration: FLOLAN is administered by continuous intravenous infusion via a central venous catheter using an ambulatory infusion pump. During dose-ranging, FLOLAN may be administered peripherally.

The ambulatory infusion pump used to administer FLOLAN should: (1) be small and lightweight, (2) be able to adjust infusion rates in 2 ng/kg/min increments, (3) have occlusion, end of infusion, and low battery alarms, (4) be accurate to ±6% of the

programmed rate, and (5) be positive pressure driven (continuous or pulsatile) with intervals between pulses not exceeding 3 minutes at infusion rates used to deliver FLOLAN. The reservoir should be made of polyvinyl chloride, polypropylene, or glass. Infusion pumps used in clinical trials were the CADD-1 HFX 5100 (Pharmacia Deltec), Walk-Med 410 C (Medfusion, Inc.), and the Auto Syringe AS2F (Baxter Health Care).

To avoid potential interruptions in drug delivery, the patient should have access to a backup infusion pump and intravenous infusion sets. A multi-lumen catheter should be considered if other intravenous therapies are routinely administered.

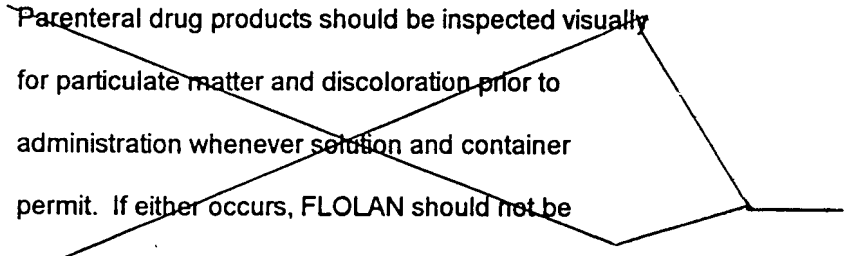
To facilitate extended use at temperatures exceeding 25°C (77°F), a cold pouch with frozen gel packs was used in clinical trials (see DOSAGE AND ADMINISTRATION: Storage and Stability). The cold pouches and gel packs used in clinical trials were obtained from Palco Labs, Palo Alto, California.

INSERT:
ambient

ADD:
Any cold pouch used must be capable of maintaining the temperature of reconstituted FLOLAN between 2° and 8°C for 12 hours.

Reconstitution: FLOLAN is only stable when reconstituted with STERILE DILUENT for FLOLAN. FLOLAN must not be reconstituted or mixed with any other parenteral medications or solutions prior to or during administration.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. If either occurs, FLOLAN should not be administered.



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A concentration for the solution of FLOLAN for acute dose ranging or chronic therapy should be selected which is compatible with the infusion pump being used with respect to minimum and maximum flow rates, reservoir capacity, and the infusion pump criteria listed above. FLOLAN, when administered chronically, should be prepared in a drug delivery reservoir appropriate for the infusion pump with a total reservoir volume of at least 100 mL. FLOLAN should be prepared using 2 vials of STERILE DILUENT for FLOLAN for use during a 24-hour period. Table 5 gives directions for preparing several different concentrations of FLOLAN:

Table 5

To make 100 mL of solution with final Concentration (ng/mL) of:	Directions:
3,000 ng/mL	Dissolve contents of one 0.5 mg vial with 5 mL of STERILE DILUENT for FLOLAN. Withdraw 3 mL and add to sufficient STERILE DILUENT for FLOLAN to make a total of 100 mL.
5,000 ng/mL	Dissolve contents of one 0.5 mg vial with 5 mL of STERILE DILUENT for FLOLAN. Withdraw entire vial contents and add sufficient STERILE DILUENT for FLOLAN to make a total of 100 mL.
10,000 ng/mL	Dissolve contents of two 0.5 mg vials each with 5 mL of STERILE DILUENT for FLOLAN. Withdraw entire vial contents and add sufficient STERILE DILUENT for FLOLAN to make a total of 100 mL.
15,000 ng/mL*	Dissolve contents of one 1.5 mg vial with 5 mL of STERILE DILUENT for FLOLAN. Withdraw entire vial contents and add sufficient STERILE DILUENT for FLOLAN to make a total of 100 mL.

*Higher concentrations may be required for patients who receive FLOLAN long-term.

More than one solution strength may be required to accommodate the range of infusions anticipated during acute dose-ranging. Generally, 3,000 ng/mL and 10,000 ng/mL are satisfactory concentrations to deliver between 2 to 16 ng/kg/min in adults. Infusion rates may be calculated using the following formula:

$$\textbf{Infusion Rate (mL/hr)} = \frac{\textbf{[Dose (ng/kg/min) x Weight (kg) x 60 min/hr]}}{\textbf{Final Concentration (ng/mL)}}$$

Tables 6 through 9 provide infusion delivery rates for doses up to 16 ng/kg/min based upon patient weight,

drug delivery rate, and concentration of the solution of FLOLAN to be used. These tables may be used to select the most appropriate concentration of FLOLAN that will result in an infusion rate between the minimum and maximum flow rates of the infusion pump and which will allow the desired duration of infusion from a given reservoir volume. Higher infusion rates, and therefore, more concentrated solutions may be necessary with long-term administration of FLOLAN.

Table 6

Infusion Rates for FLOLAN at a Concentration of 3,000 ng/mL

Patient Weight (kg)	Dose or Drug Delivery Rate (ng/kg/min)							
	2	4	6	8	10	12	14	16
	Infusion Delivery Rate (mL/hr)							
10	—	—	1.2	1.6	2.0	2.4	2.8	3.2
20	—	1.6	2.4	3.2	4.0	4.8	5.6	6.4
30	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6
40	1.6	3.2	4.8	6.4	8.0	9.6	11.2	12.8
50	2.0	4.0	6.0	8.0	10.0	12.0	14.0	16.0
60	2.4	4.8	7.2	9.6	12.0	14.4	16.8	19.2
70	2.8	5.6	8.4	11.2	14.0	16.8	19.6	22.4
80	3.2	6.4	9.6	12.8	16.0	19.2	22.4	25.6
90	3.6	7.2	10.8	14.4	18.0	21.6	25.2	28.8
100	4.0	8.0	12.0	16.0	20.0	24.0	28.0	32.0

Table 7

Infusion Rates for FLOLAN at a Concentration of 5,000 ng/mL

Patient Weight (kg)	Dose or Drug Delivery Rate (ng/kg/min)							
	2	4	6	8	10	12	14	16
	Infusion Delivery Rate (mL/hr)							
10	—	—	—	1.0	1.2	1.4	1.7	1.9
20	—	1.0	1.4	1.9	2.4	2.9	3.4	3.8
30	—	1.4	2.2	2.9	3.6	4.3	5.0	5.8
40	1.0	1.9	2.9	3.8	4.8	5.8	6.7	7.7
50	1.2	2.4	3.6	4.8	6.0	7.2	8.4	9.6
60	1.4	2.9	4.3	5.8	7.2	8.6	10.1	11.5
70	1.7	3.4	5.0	6.7	8.4	10.1	11.8	13.4
80	1.9	3.8	5.8	7.7	9.6	11.5	13.4	15.4
90	2.2	4.3	6.5	8.6	10.8	13.0	15.1	17.3
100	2.4	4.8	7.2	9.6	12.0	14.4	16.8	19.2

Table 8

Infusion Rates for FLOLAN at a Concentration of 10,000 ng/mL

Patient Weight (kg)	Dose or Drug Delivery Rate (ng/kg/min)						
	4	6	8	10	12	14	16
	Infusion Delivery Rate (mL/hr)						
20	—	—	1.0	1.2	1.4	1.7	1.9
30	—	1.1	1.4	1.8	2.2	2.5	2.9
40	1.0	1.4	1.9	2.4	2.9	3.4	3.8
50	1.2	1.8	2.4	3.0	3.6	4.2	4.8
60	1.4	2.2	2.9	3.6	4.3	5.0	5.8
70	1.7	2.5	3.4	4.2	5.0	5.9	6.7
80	1.9	2.9	3.8	4.8	5.8	6.7	7.7
90	2.2	3.2	4.3	5.4	6.5	7.6	8.6
100	2.4	3.6	4.8	6.0	7.2	8.4	9.6

Table 9

Infusion Rates for FLOLAN at a Concentration of 15,000 ng/mL							
Patient	Dose or Drug Delivery Rate (ng/kg/min)						
Weight (kg)	4	6	8	10	12	14	16
	Infusion Delivery Rate (mL/hr)						
30	—	—	1.0	1.2	1.4	1.7	1.9
40	—	1.0	1.3	1.6	1.9	2.2	2.6
50	—	1.2	1.6	2.0	2.4	2.8	3.2
60	1.0	1.4	1.9	2.4	2.9	3.4	3.8
70	1.1	1.7	2.2	2.8	3.4	3.9	4.5
80	1.3	1.9	2.6	3.2	3.8	4.5	5.1
90	1.4	2.2	2.9	3.6	4.3	5.0	5.8
100	1.6	2.4	3.2	4.0	4.8	5.6	6.4

Storage and Stability: Unopened vials of FLOLAN are stable until the date indicated on the package when stored at 15° to 25°C (59° to 77°F) and protected from light in the carton. Unopened vials of STERILE DILUENT for FLOLAN are stable until the date indicated on the package when stored at 15° to 25°C (59° to 77°F).

Prior to use, reconstituted solutions of FLOLAN must be protected from light and must be refrigerated at 2° to 8°C (36° to 46°F) if not used immediately. **Do not freeze reconstituted solutions of FLOLAN. Discard any reconstituted solution that has been frozen.**

ADD:
Discard any reconstituted solution if it has been refrigerated for more than 48 hours.

During use, a single reservoir of reconstituted solution of FLOLAN can be administered at room temperature for a total duration of 8 hours, or it can be administered up to 24 hours with the use of two frozen 6-oz gel packs in a cold pouch. When stored or in use, reconstituted FLOLAN must be insulated from temperatures greater than 25°C (77°F) and less than 0°C (32°F), and must not be exposed to direct sunlight.

INSERT:
used with a cold pouch and

Use at Room Temperature: Prior to use at room temperature, 15° to 25°C (59° to 77°F), reconstituted solutions of FLOLAN may be stored refrigerated at 2° to 8°C (36° to 46°F) for no longer than 40 hours. When administered at room temperature, reconstituted solutions may be used for no longer than 8 hours. This 48-hour period allows the patient to reconstitute a 2-day supply (200 mL) of FLOLAN. Each 100 mL daily supply may be divided into three

equal portions. Two of the portions are stored refrigerated at 2° to 8°C (36° to 46°F) until they are used.

Use with a Cold Pouch: Prior to infusion with the use of a cold pouch, solutions may be stored refrigerated at 2° to 8°C (36° to 46°F) for up to 24 hours. When a cold pouch is employed during the infusion, reconstituted solutions of FLOLAN may be used for no longer than 24 hours. The gel packs should be changed every 12 hours.

ADD:
Reconstituted solutions may be kept at 2°C to 8°C (36° to 46°F), either in refrigerated storage or in a cold pouch or a combination of the two, for no more than 48 hours.

HOW SUPPLIED: FLOLAN for Injection is supplied as a sterile freeze-dried powder in 17 mL flint glass vials with gray butyl rubber closures, individually packaged in a carton.

ADD NEW PARAGRAPH:
Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. If either occurs, reconstituted FLOLAN should not be administered.

17 mL vial containing epoprostenol sodium equivalent to 0.5 mg (500,000 ng), carton of 1, (NDC 0081-0460-01).

17 mL vial containing epoprostenol sodium equivalent to 1.5 mg (1,500,000 ng), carton of 1, (NDC 0081-0464-01).

Store the vials of FLOLAN at 15° to 25°C (59° to 77°F). Protect from light.

The STERILE DILUENT for FLOLAN is supplied in 50 mL flint glass vials with fluororesin faced butyl rubber closures.

50 mL vial of STERILE DILUENT for FLOLAN, tray of 4 (NDC 0081-0462-01).

Store the vials of STERILE DILUENT for FLOLAN at 15° to 25°C (59° to 77°F). DO NOT FREEZE.

Caution: Federal law prohibits dispensing without prescription.

U.S. Patent Nos. 4335139, 4539333, and 4883812 (Use Patent)
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EXHIBIT 6

U.S. PATENT 4,883,812

United States Patent [19]

Moncada

[11] **Patent Number: 4,883,812**

[45] **Date of Patent: * Nov. 28, 1989**

[54] **TREATMENT OF HYPERTENSION USING PROSTACYCLIN**

[75] Inventor: **Salvador Moncada**, West Wickham, England

[73] Assignee: **Burroughs Wellcome Co.**, Research Triangle Park, N.C.

[*] Notice: The portion of the term of this patent subsequent to Sep. 3, 2002 has been disclaimed.

[21] Appl. No.: **237,987**

[22] Filed: **Aug. 29, 1988**

Related U.S. Application Data

[63] Continuation of Ser. No. 712,788, Mar. 18, 1985, which is a continuation of Ser. No. 795,524, May 10, 1977, Pat. No. 4,539,333.

[30] **Foreign Application Priority Data**

Aug. 17, 1976 [GB] United Kingdom 34151

Sep. 3, 1976 [GB] United Kingdom 36547

[51] **Int. Cl.**[4] **A61K 31/557; A61K 31/34**

[52] **U.S. Cl.** **514/469**

[58] **Field of Search** 514/469

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,338,325	7/1982	Johnson et al.	514/469
4,430,340	7/1984	Cho	514/469
4,499,293	2/1985	Johnson et al.	549/465
4,539,333	9/1985	Moncada	514/469

Primary Examiner—Mary C. Lee
Assistant Examiner—Bernard I. Dentz
Attorney, Agent, or Firm—Donald Brown

[57] **ABSTRACT**

Prostacyclin, its salts biosynthesis and synthesis thereof, pharmaceutical formulations containing them, and their use in medicine.

4 Claims, No Drawings

TREATMENT OF HYPERTENSION USING PROSTACYCLIN

This is a continuation of co-pending application Ser. No. 712,788 filed on Mar. 18, 1985 which is a continuation of Ser. No. 795,524 filed on May 10, 1977 U.S. Pat. No. 4,539,333.

This invention relates to the extraction, isolation and synthesis of a prostaglandin derivative, its use as a chemical intermediate, formulations containing it, and its use in medicine.

Prostaglandin endoperoxides (PGG_2 and PGH_2) are generated from arachidonic acid by a membrane-bound cyclo-oxygenase enzyme system, described by Hamberg and Samuelsson (Biochem, Biophys. Acta. 326, 448–461,1974) and are subsequently transformed to $PGF_{2\alpha}$, PGE_2, PGD_2, or Thromboxane A_2. Thromboxane A_2 shares with the prostaglandin endoperoxides the important biological properties of contracting strips of rabbit aorta and aggregating blood platelets.

The Applicants have now found that microsomes derived from a variety of mammalian tissues catalyse the enzymic transformation of the prostaglandin endoperoxides to a prostaglandin derivative (hereinafter referred to as Prostacyclin which does not contact strips of rabbit aorta, relaxes strips of rabbit coeliac mesenteric and coronary arteries, has a potent anti-aggregatory action on blood platelets, is a strong vasodilator in whole animals and has other properties described hereinafter.

Microsomes derived from rabbit or pig blood vessels such as veins and arteries, and rat stomach fundus produce about 80–90% conversion of the prostaglandin endoperoxides. Microsomes derived from rabbit lung tissue and rat pyloric tissue produce 25% conversion of the prostaglandin endoperoxides, whereas those derived from rat kidney, brain, spleen, liver, heart and seminal vesicle tissues produce 5% or less conversion of the prostaglandin endoperoxides.

Prostacyclin is unstable at room temperature in aqueous medium, having a half-life of approximately 10 mins., but its anti-aggregatory activity can be preserved for several days by dissolving the substance in aqueous alkali or in dry acetone and storing at −20° C. On average prostacyclin is 10–40 times more potent as an anti-aggregatory agent than PGE_1 and 5–20 times more potent than PGD_2, itself a potent inhibitor of platelet aggregation. Prostacyclin also interrupts and reverses the presence of platelet aggregation.

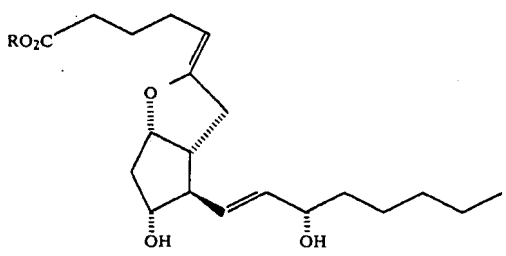
Prostacyclin may be prepared biosynthetically by incubating PGG_2 or PGH_2 with aortic microsomes in a suitable buffer solution such as Tris buffer, for approximately 2 minutes at a temperature in the region of 22° C. Conversion of the prostaglandin endoperoxides is approximately 85%.

Extraction of Prostacyclin is achieved by the addition of cold (0° C.) dry diethyl ether to the incubation mixture. The addition of cold ether stops the enzyme reaction and Prostacyclin enters the ether phase which may be separated from the aqueous phase. Evaporation of the ether by standard techniques such as bubbling nitrogen through the solution results in the Prostacyclin being left as a residue which may subsequently be resuspended in an aqueous solution for further examination or dissolved in anhydrous acetone and stored at a temperature in the region of −20° C. for future use.

The aortic microsomes employed in the incubation mixture may be extracted from pig or rabbit aortas. Aortas may be frozen solid preferably by dropping them in liquid nitrogen, and then crushed to form a powder which is resuspended in a suitable buffer solution and subsequently homogenized. The homogenate may then undergo sequential centrifugation so as to isolate the microsomal fraction which may be resuspended in deionized water and lyophilized. The incubation mixture was shown to have an immediate anti-aggregatory effect by monitoring the aggregation of human blood platelets in a Born aggregometer.

Prostacyclin may be prepared using other tissues identified above in substantially a similar manner. Prostacyclin formed in such an incubation mixture appears to be different from the other products of PG endoperoxides so far described. Its biological properties on the isolated tissues, its instability and its potent anti-aggregatory activity show that Prostacyclin is not being PGE_2 or $PGF_{2\alpha}$. The presence of prostaglandin D_2 isomerase in homogenates of several tissues has been described. As prostacyclin is unstable and is a more potent anti-aggregatory agent than PGD_2, it cannot be regarded as PGD_2. Furthermore, PGD_2 isomerase is present in the 100,000 g. supernatant, a fraction that did not produce Prostacyclin from PG endoperoxides. Moreover, PGD_2 isomerase needs glutathione as a co-factor and the incubations were carried out in the absence of cofactors. PGE_2, $PGF_{2\alpha}$ and PGD_2 were not substrates for aortic microsomes and therefore 15-keto PGs and other products of prostaglandin catabolism could not be considered as Prostacyclin. Prostacyclin is also unlikely to be a known 15-hydroperoxy PG, firstly because 15-hydroperoxy PGE_2 has a contractile activity on rabbit aortic strip and secondly the product(s) of the spontaneous decay of Prostacyclin when bioassayed did not behave like PGE_2, $PGF_{2\alpha}$, or PGD_2. As Prostacyclin has an anti-aggregatory activity it cannot be identical to Thromboxane A_2 or B_2 as they are pro-aggregatory substances.

Further studies have shown that Prostacyclin has the chemical structure shown in formula (I) (R is hydrogen) (see Johnson et al., Prostaglandins, 12/6, 915–928, 1976).

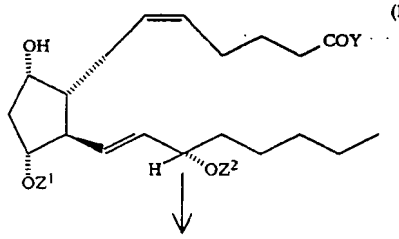


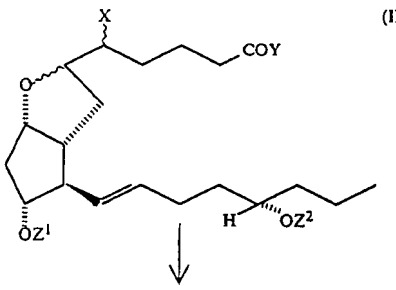
(I)

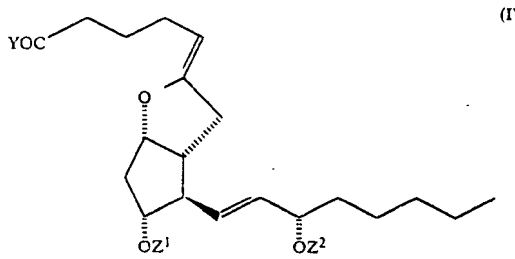
By the present invention we provide the compounds of formula (I) wherein R is hydrogen or a pharmacologically acceptable cation (hereinafter referred to as Prostacyclin and salts thereof). Salts of Prostacyclin include alkali metal salts, alkaline earth metal salts and salts of organic bases.

Salts of Prostacyclin may be synthesised from a compound of formula (II) wherein either or both of Z^1 and Z^2 is hydrogen or a blocking group such as acyl, or trialkylsilyl (for example trimethylsilyl). Oxidative attack by iodine or potassium triiodide in the presence of

a metal bicarbonate at the 5,6-double bond of a compound of formula (II) with simultaneous or subsequent cyclisation involving the 9-hydroxy group produces a compound of formula (III). Upon treatment with a suitable base such as an organic base or a metal alkoxide, a compound of formula (III) may then be dehydrohalogenated, resulting in the introduction of a 5,6-double bond. This reaction sequence is illustrated in the following reaction scheme:

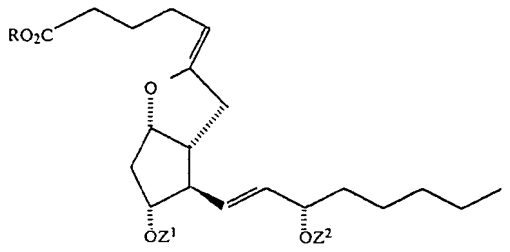






wherein Y is OH, NHR^1 or OR^1, R^1 being alkyl of 1 to 4 carbon atoms or a cation; X is iodo or bromo; and Z^1 and Z^2 are as defined above.

Included in compounds of formula (IV) are compounds of formula



wherein R is hydrogen or a pharmacologically acceptable cation and each of Z^1 and Z^2 is hydrogen or a blocking group.

When Z^1 and/or Z^2 in formulae (II), (III) and (IV) are blocking groups, the resulting blocked derivatives of the compounds of formula (I) may be converted to the corresponding compounds of formula (I) by methods known in the art, for example base hydrolysis.

Prostacyclin salts may be prepared by treating an ester thereof (formula (I) : R is alkyl) for example the methyl ester, with a strong base such as sodium hydroxide in a suitable solvent and lyophilising the resulting reaction mixture. Prostacyclin itself may be conveniently prepared by base hydrolysis of its corresponding esters or amides in the presence of an equivalent amount of a caustic alkali in an aqueous alcohol or an aqueous tetrahydrofuran medium, and extracted into an organic solvent at low temperature.

Prostacyclin and its salts are useful as intermediates in the synthesis of prostaglandin analogues, and exhibit a potent anti-aggregatory action on blood platelets, and therefore have a particular utility in the treatment and/or prophylaxis of mammals as anti-thrombotic agents.

They are also useful in mammals, including man, to reduce and control excessive gastric secretion, thereby reducing or avoiding gastrointestinal ulcer formation, and accelerating the healing of such ulcers and lesions already present in the gastrointestinal tract.

Prostacyclin and its salts further exhibit vasodilatory action on blood vessels and therefore have a particular utility as anti-hypertensives for the treatment of high blood pressure in mannals, including man. Use as an antihypertensive may be accomplished by administering a pharmaceutical solution containing the anion of (5Z)-5,6-Didehydro-9-deoxy-6,9-epoxy prostaglandin $F_{1\alpha}$ Platelets can be assimilated into the vascular endothelium or even incorporated into endothelial cells. Biochemical co-operation between platelets and vascular endothelium in the generation of Prostacyclin contributes to the repair of vascular endothelium, and Prostacyclin and its salts have a further utility in the promotion of wound healing in mammals, including man.

Prostacyclin and its salts may be used whenever it is desired to inhibit platelet aggregation, to reduce the adhesive character of platelets, and to treat or prevent the formation of thrombi in mammals, including man. For example, they may be used in the treatment and prevention of myocardial infarcts, λ in the treatment of peripheral vascular disease λ to treat and prevent post-operative thrombosis, to promote patency of vascular grafts following surgery, and to treat complications of arteriosclerosis and conditions such as atherosclerosis, blood clotting defects due to lipemia, and other clinical conditions in which the underlying etiology is associated with lipid imbalance or hyperlipidemia.

They may also be used as additives to blood, blood products, blood substitutes, and other fluids which are used in artificial extra-corporeal circulation and perfusion of isolated body portions, e.g., limbs and organs, whether attached to the original body, detached and being preserved or prepared for transplant, or attached to a new body. During these circulations and perfusions, aggregated platelets tend to block the blood vessels and portions of the circulation apparatus. This blocking is avoided by the presence of Prostacyclin. For this purpose, Prostacyclin or its salts may be added gradually or in single or multiple portions to the circulating blood, to the blood of the donor animal, to the perfused body portion, attached or detached to the recipient, or to two or all of those at a total steady state dose of 0.001 to 10 mg., per liter of circulating fluid. It is especially useful to use Prostacyclin in laboratory animals, e.g. cats, dogs, rabbits, monkeys and rats, for

these purposes in order to develop new methods and techniques for organ and limb transplants.

The amount of prostacyclin or a salt thereof required (hereinafter referred to as the active ingredient) for therapeutic effect will vary with the route of administration. In general a suitable dose for a mammal will lie in the range of 0.01 to 200 mg. per kilogram bodyweight, conveniently 0.01 to 10 mg per kilogram.

While it is possible for the active ingredient to be administered as the raw chemical it is preferable to present it as a pharmaceutical formulation. Such formulations are preferably non-aqueous and non-hydroxylic in nature, but alkaline aqueous solutions may be used. Unit doses of a formulation contain between 0.5 mg. and 1.5 g of the active ingredient.

Such formulations, both for veterinary and for human medical use, of the present invention comprise the active ingredient, as above defined, together with one or more acceptable carriers therefor and optionally other therapeutic ingredeints. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for parenteral (including subcutaneous, intramuscular and intravenous) administrationn which must of course be sterile.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

According to the present invention there are therefore provided:

(a) the compounds of formula (I) and derivatives thereof blocked in the 11- and 15- positions;

(b) the preparation of Prostacyclin comprising:
  (i) incubation of microsomes of fresh mammalian tissue with a prostaglandin endoperoxide, and the extraction of prostacyclin from the incubation mixture into an organic solvent; or
  (ii) its synthesis as hereinbefore described;

(c) Prostacyclin when obtained by the process described in paragraph (b);

(d) a pharmaceutical formulation containing prostacyclin;

(e) the preparation of such pharmaceutical formulations;

(f) method for the treatment or prophylaxis of thrombosis in a mammal or mammalian tissues, including man,comprising the administration of a non-toxic, prophylactic anti-thrombotic amount of a compound of formula (I).

(g) method for inducing vasodilation in a mammal, including man,comprising the administration of a non-toxic, vasodilatory amount of a compound of formula (I);

(h) method for the prophylaxis or treatment of gastric lesions in a mammal, including man comprising the administration of a non-toxic, prophylactic or therapeutic amount of a compound of formula (I);

(i) (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxy prostaglandin-$F_{1\alpha}$ and its salts

(j) method for the promotion of wound healing in a mannal, including man,comprising the administration of a non-toxic wound-treatment amount of a compound of formula (I).

The following examples are provided by way of an illustration of the present invention and should in no way be construed as constituting a limitation thereof.

EXAMPLE 1

Preparation of Prostacyclin

Pig aortas were stripped of adventitia, snap frozen in liquid nitrogen, crushed into a fine powder, resuspended in 0.05 M Tris buffer (pH 7.5) (1:4, w:v) and homogenised at high-speed in a Polytron (KIMENATIC, LUCERNE, SWITZERLAND) homogenizer. The homogenate was centrifuged at 1000 x g for 15 minutes and the resulting supernatant centrifuged again at 10,000 x g for 5 minutes. The 10,000 x g pellet was discarded, while the pellet obtained after centrifugation of the supernatant at 100,000 g for 60 minutes was resuspended in deionized water and lyophilized. An average yield of 150 mg. of aortic microsomal powder (51% protein) per 100 g. of aortic tissue was obtained.

Aortic microsomal powder (5 mg) was incubated with 1 μg of prostalgandin endoperoxide (PGG_2 or PGH_2) in 0.05M Tris buffer (pH 7.5) (1 ml.) for 2 minutes at 22° C. Enzyme activity was estimated by direct bioassay of the incubation mixture. After incubation of 2 minutes at 22° C. all prostaglandin endoperoxide activity was lost indicated by a lack of contraction of rabbit aorta strips when assayed by the cascade superfusion technique of Vane (Br. J. Pharmac. 23, 369–373, 1964 indicating 100% conversion of PGG_2 or PGH_2.

The Prostacyclin was extracted by the addition of cold dry diethyl ether (1 ml) to the incubation mixture which also stopped the enzymic reaction. The prostacyclin entered the ether phase, which was subsequently separated from the aqueous phase. The ether was evaporated by bubbling nitrogen through it leaving the Prostacyclin which was either dissolved in ice-cold 0.05M Tris buffer (0.5–1 ml.) and immediately used for platelet aggregation studies or was dissolved in anhydrous acetone (1 ml.) and stored at −20° C. for future use.

The anti-aggregatory activity of the extracted Prostacyclin disappeared on bioling (15 seconds) or on standing at 22° C. for 20 minutes. The anti-aggregatory activity of Prostacyclin could be preserved for several days by dissolving the substance in dry acetone and storing at −20° C.

By using rabbit aortas in a similar experiment the same results were obtained.

EXAMPLE 2

Aggregation of platelets in 1 ml. of fresh human platelet rich plasma (PRP) was monitored in a Born aggregometer. An immediate anti-aggregatory effect of the fresh reaction mixture of Example 1 of aortic microsomes with PGH_2 or PGG_2 was observed. The lowest anti-aggregatory concentration was obtained with samples containing 0.5–5 ng. Prostacyclin/ml.

This activity disappeared after leaving the incubation mixture for 20 minutes at 22° C. or by bioling for 15 seconds. Aortic microsomes alone (50 μg/ml) could induce aggregation in some PRP. The products of spontaneous degradation of PGG_2 (100 ng/ml) had no anti-aggregatory activity.

Diethyl ether extracts of Prostacyclin also inhibited platelet aggregation induced by arachidonic acid and PGG_2. The effective anti-aggregatory concentration of Prostacyclin after storate in ether was from 1 to 10 ng/ml.

EXAMPLE 3

Prostacyclin was injected intravenously and intra-aortically into normotensive anaethetised rats and the blood pressure and heart rate recorded. Results shows prostacyclin to be a power vasodepressor having 5 times the potency of PGE_2.

EXAMPLE 4

Prostacyclin was found to relax spirally cut strips of coronary artery from the ox. The effect is dose dependent and relaxation can be seen with doses as low as 20 nanograms per 5 ml bath. In isolated hearts from rabbits perfused at constant flow rate by the Langendorf technique, Prostacyclin produced coronary vasodilation.

Prostacyclin relaxed strips of coeliac and mesenteric arteries, but was less potent than PGE_2.

EXAMPLE 5

Prostacyclin was shown to inhibit the formation of gastric lesions induced in rats by indomethacin at doses from 62.5 to 250μg upon subcutaneous injection.

EXAMPLE 6

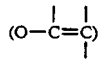
A stirred solution of $PGF_{2\alpha}$ methyl ester (50 mg) in ether (1 ml) was treated with sodium bicarbonate (115.0 mg; 10 molecular equivalents) and water (1 ml) and then dropwise during 2 hours with aqueous potassium triiodide (0.7 molar; 0.261 ml). After stirring overnight, the reaction mixture was shaken with ether and aqueous sodium thiosulphate; the etheral phase was separated, washed with water, dried with magnesium sulphate, and evaporated to leave a yellow gum of 5ξ-iodo-9-deoxy-6ξ,9α-epoxyprostaglandin $F_{1\alpha}$ methyl ester.

A solution of 5ξ-iodo-9-deoxy-6ξ,9α-epoxyprostaglandin $F_{1\alpha}$ methyl ester (100 mg.) in methanolic sodium methoxide prepared from sodium (46 mg.) and dry methanol (0.70 ml.) was set aside under dry nitrogen for 5 hours, then freed from solvent in high vacuum. The residual amorphous solid was washed with benzene, set aside in the air overnight, and stirred with N aqueous sodium hydroxide (0.5 ml.) to give a suspension of colourless fine needles. The crystals were collected, washed with a few drops of N aqueous sodium hydroxide, and dried in the air to give the sodium salt of 9-deoxy-6,9α-epoxy-Δ^5-prostaglandin $F_{1\alpha}$. The inhibition of arachidonic acid-induced aggregation of human platelets at a concentration of 0.2 ng./ml. by this salt and its instability in water at acid pH, together with further evidence, is compatible with assignation of the configuration (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxy-prostaglandin $F_{1\alpha}$ sodium salt.

The high-resolution ^{13}C n.m.r. spectrum of a solution of the crystals in dimethyl sulphoxide-d_6 showed the expected 20 resonances whose chemical shifts were entirely consistant with the chemical structure established for Prostacyclin. No impurity peaks were detected.

EXAMPLE 7

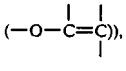
5ξ-Iodo-9-deoxy-6ξ,9α-epoxyprostaglandin $F_{1\alpha}$ methyl ester (500 mg) was stirred with methanolic NaOMe prepared from Na (0.23 g., 10 equivs.) and MeOH (3.5 ml.) under N_2 at room temperature overnight; 1N aq. NaOH (2.5 ml.) was added to the yellow reaction solution to bring about hydrolysis of the ester moiety and, after 2 hours, the methanol was evaporated in vacuo at room temperature. The residual aqueous solution gave rise spontaneously to a mass of colourless fine needles of the desired sodium salt (formula (I): R=Na) which was cooled (0°), collected, washed sparingly with 1N aq. NaOH, air-dried, and stored in a stoppered tube; this salt (383mg.) had λ(KBr disc) νmax 1692 cm^{-1}



and twenty ^{13}C resonances only were observed (at 182.7 (C-1), 158.2 (C-6), 140.0 and 134.3 (C-13,14), 100.7(C-5), 87.5(C-15), 80.6 and 75.5(C-9,11), 58.0(C-12), 49.0, 45.8, 42.4, 41.9, 37.5, 35.8 (C-18), 31.6, 29.9, 29.3, 26.7(C-19), and 18.4(C-20) ppm from TMS in DMSO-d_6). The product, sodium (5Z)- 5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin $F_{1\alpha}$ (syn. sodium prostacyclin), thus obtained complete inhibited arachidonic acid-induced platelet aggregation (human platelet-rich plasma) at 1 ng./ml. and its profile of biological activity on the rabbit aorta, rabbit coeliac artery, rat stomach strip and rat colon conformed with that of sodium prostacyclin obtained by biosynthesis. After air-drying, the salt has a surface coating of sodium carbonate (ca 3.5% by weight) which protects the vinyl ether moiety against carbondioxide catalysed hydrolysis.

EXAMPLE 8

5ξ-Iodo-9-deoxy-6ξ,9α-epoxyprostaglandin $F_{1\alpha}$ methyl ester was treated with 1,5-diazabicyclo-5-nonene (DBN) at room temperature in the absence of a solvent for a few hours. The DBN and hydrogen iodide were conveniently removed by adsorption on to a column of SiO_2, prepared from a suspension of SiO_2 in EtOAc/Et_3N 50:1, and the vinyl ether was eluted with the same solvent system. I.R. spectroscopy (thin film, ν_{max} 1738 (CO_2Me) and 1696 cm^{-1}



^1H n.m.r. in C_6D_6-Et_3N, 19:1 (δ4.22, triplet of triplets[12], J6.9 and 1.0 Hz (C-5 vinyl proton)), and ^{13}C n.m.r. in C_6D_6-Et_3N, 19.1 (distinctive features were resonances at 159.8 (C-1), 155.8 (C-6), 137.2 and 130.6 (C-13,14), 95.3 (C-5), 84.1 (C-15) 77.3 and 72.2 (C-9,11) and 51.1. (Me ester)p.p.m. from TMS).

The vinyl ether (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin methyl ester, was hydrolysed with aqueous sodium hydroxide to give sodium prostacyclin (formula (I): R=sodium).

What we claim is:

1. A method of treating a mammal for hypertension which comprises parenterly administering an effective anti-hypertensive amount of prostacyclin to said mammal.

2. The method of claim 1 in which the prostacyclin is administered by injection as part of a solution.

3. A method of treating a mammal for hypertension which comprises parenterally administering an effective antihypertensive amount of prostacyclin anion in a liquid to said mammal.

4. The method of claim 3 in which the prostacyclin anion is administered by injection.

* * * * *

EXHIBIT 7

DUE DILIGENCE LOG
for IND 16,459 and NDA 20-444

08–03–78 Letter to FDA in reference to the BW–FDA meeting scheduled for 1:00 pm on 8/29/78 to discuss plans for our submission of an IND for Prostacyclin (PGI_2), advising that the meeting is primarily intended to share with FDA the current state of our research effort in this area and secondly, to discuss future plans to bring the drug into use in a variety of disease states.

05–30–79 Submitted a "Notice of Claimed Investigational Exemption for a New Drug."

06–06–79 Letter from FDA acknowledging receipt of our 5/30/79 Notice of Claimed Investigational Exemption for a New Drug.

10–20–79 Submitted an amendment to provide for manufacturing and control changes.

01–04–80 Telephone call to FDA to report an emergency shipment of prostacyclin to Duke University for an infant with persistent pulmonary hypertension in a newborn.

07–02–80 Telephone call to FDA to inform them of the deaths of a newborn infant female, treated on an emergency basis at Duke University Medical Center, July 1 and 2, 1980, for persistent pulmonary hypertension.

07–09–80 Telephone call to FDA to discuss the infant with persistent pulmonary hypertension who was treated with prostacyclin at Duke University Medical Center.

07–15–80 Amended our IND to provide for the clinical study "Pilot Study of Epoprostenol (Prostacyclin Sodium) in the the Treatment of Persistent Pulmonary Hypertension of the Newborn (PPHN) In Neonates Unresponsive to Ventilatory Therapy," to be conducted by George Brumley, M.D.

07–23–80 Submitted Progress Report.

09–15–80 Amended our IND to provide for the clinical study "Pilot Study of Epoprostenol (Prostacyclin Sodium) In the Treatment of Persistent Pulmonary Hypertension of the Newborn (PPHN) In Neonates Unresponsive to Ventilatory Therapy," to be conducted by Herbert Harned, Jr., M.D.

10–20–80 Amended our IND to provide for the clinical study "Pilot Study of Epoprostenol (Prostacyclin Sodium) In the Treatment of Primary Pulmonary Hypertension (PPH) in Adults," to be conducted by Lewis Rubin, M.D., and Bertron Groves, M.D.

11–12–80 Submitted an amendment to provide for a revision in the pH of the solution of Prostacyclin for freeze drying from pH 10.5 ± 0.05 to pH 11.4 ± 0.05.

11–21–80 Amended our IND to allow for the manufacture of the diluent buffer for the injection at two alternate sites, The Wellcome Foundation, Ltd., Beckenham, U.K., and Glaxo Biologicals Speke, Near Liverpool, U.K.

01–28–81 Telephone call to FDA to report an instability problem with prostacyclin; advised FDA that a more detailed letter is forthcoming.

04–20–81 Letter to FDA in reference to the 1/28/81 BW–FDA telephone conversation advising FDA of a stability problem with prostacyclin, forwarding a report prepared by the Wellcome Foundation, Ltd., which fully describes the background of the stability problem.

04–20–81 Meeting with FDA to discuss (1) formal notification to FDA of the problem of loss of potency in one lot of drug, our investigation, and our conclusion (2) discussion of a preliminary review of our IND by Dr. Weiss.

05–28–81 Telephone conversation with FDA regarding their interest in prostacyclin as a diagnostic tool in determining the reversibility of pulmonary hypertension.

06–02–81 In reference to our letter of 4/20/81 concerning a stability problem with prostacyclin, submitted original graphs of the results of the Pyrogallol experiment to show the oxygen levels.

08–31–81 Submitted Progress Report.

11–02–81 Telephone conversation with the FDA to request permission to administer prostacyclin to a patient with primary pulmonary hypertension, entered in Dr. Bertram Groves clinical study.

11–05–81 Letter to FDA regarding recent observations by the Upjohn Company of alterations in bone growth in dogs and neonates following administration of PGE_1, PGE_2, and PGF_2.

01–12–82 Telephone call to Dr. Eugenia Triantas (FDA) to request permission to treat patient with pulmonary hypertension.

01–18–82 Telephone call to FDA to request permission to treat a patient with primary pulmonary hypertension.

02–15–82 Submitted an amendment to our IND to provide for the use of a 0.22 um sterile membrane filter.

06–24–82 Amended our IND to provide for a clinical study entitled "evaluation of Epoprostenol Sodium and Subsequent Therapy in the Treatment of Primary Pulmonary Hypertension (PPH) in Adults," to be conducted by Bertron Groves, M.D.

07–27–82 Amended our IND to provide for the clinical study entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH).

07–27–82 In addition, we wish to amend our IND to provide for the clinical study entitled "Evaluation of Epoprostenol sodium and Subsequent Therapy in the Treatment of Primary Pulmonary Hypertension (PPH) in Adults," to be conducted by Lewis J. Rubin, M.D.

08–10–82 Amended our IND to provide for the emergency use of Prostacyclin Sodium Salt by Bertron Groves, M.D., of University of Colorado School of Medicine, Denver, Colorado and Hugo D. Mortenegro, M.D., Cleveland VA Medical Center, Cleveland, Ohio, in the treatment of patients with primary pulmonary hypertension.

09–11–82 Submitted Progress Report

09–02–82 Amended our IND to register Robert Mellins, M.D., as an investigator for the clinical study entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)," submitted to FDA 7–27–82.

09–07–82 Telephone conversation with Doralie Segal of FDA regarding Dr. Herbert B. Hectman's clinical study.

10–01–82 Amended our IND to provide for an amendment to the clinical protocol entitled "Evaluation of Epoprostenol Sodium and Subsequent Therapy in the Treatment of Primary Pulmonary Hypertension (PPH) in Adults," being conducted by Lewis J. Rubin, M.D., and Bertron Groves, M.D., submitted 8–27–82 and 6–24–82, respectively.

04–07–83 Amended our IND to register Syed Mohiuddin, M.D., as an investigator for the clinical study (21–02) entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)," submitted to FDA 7–27–82.

05–17–83 Amended our IND to provide for revisions to the clinical study 21 entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension," being conducted by Robert Mellins, M.D. and Syed Mohiuddin, M.D.

10–17–83 Amended IND to register Bertron Groves, M.D. as investigator for the study entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension." Study No. 21–05.

12–05–83 Amended IND to provide for the following registration of Lewis J. Rubin, M.D. as an investigator for the clinical study entitled "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)," submitted to FDA on July 27, 1982, and amended on May 17, 1983.

04–25–84 Amended IND to provide for registration of Kenneth Moser, M.D. as an investigator for clinical study 21, "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)," submitted 7/27/82.

01–27–84 Submitted an amendment to our IND to provide for an updating of the synthetic route for the new drug substance.

03–15–84 Submitted a copy of the minutes of the End–of–Phase II Meeting held with FDA 11/21/83, and a copy of Technical Report on the 30–day dog study, entitled "Formation and Reversibility of Hematological Changes in Beagle Dogs," conducted by the Upjohn Company.

07–09–84 Submitted Progress Report.

07–10–84 Amended IND to provide for clinical study 29, "Evaluation of Epoprostenol Sodium Effects on Pulmonary Vascular Resistance, Pulmonary Function and Exercise Tolerance in Adult Patients With CorPulmonate from Chronic Obstructive Pulmonary Disease," to be conducted by Lewis J. Rubin, M.D. In addition, we wish to amend IND to provide for a change in prinicipal investigator from Richard Foley, M.D. to Catherine Thompson, M.D. for the following clinical studies:

11–07–84 Amended IND to provide for:

> Amendment of clinical study 29, "Evaluation of Epoprostenol Sodium on Pulmonary Vascular Resistance, Pulmonary Function, and Exercise Tolerance in Adult Patients with Cor Pulmonale from Chronic Obstructive Pulmonary Disease," being conducted by Lewis Rubin, M.D., to allow additional platelet aggregation tests. The protocol for this study and a completed Form FD 1572/1573 for Dr. Rubin were submitted to FDA on July 10, 1984.

02–07–85 Amended IND to provide for revisions to clinical study 29, being conducted by Lewis Rubin, M.D. (Protocol submitted to FDA 7–10–84 and amended 11–7–84).

05–15–85 and 05–24–85 Conversations with FDA regarding primary pulmonary hypertension (PPH) indication for prostacyclin requesting additional data.

08–14–85 Submitted Progress Report.

08–15–85 Amended IND to register Robyn Barst, M.D. as an investigator for clinical study 21, "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)," submitted to FDA on July 27, 1982, and amended on May 17, 1983.

12–24–85 Amended our IND to register Jeffrey Dunn as an investigator for clinical study 21, submitted to FDA on July 27, 1982, and amended on May 17, 1983.

02–27–86 Amended our IND to register Lewis J. Rubin, M.D., as investigator for clinical study 29, sites 03 and 04, submitted to FDA on 7/10/84, and amended on 11/7/84 and 2/7/85.

04–28–86 Amended our IND to provide for a change in principal investigator for study 21, submitted to FDA on 07/27/82 and amended on 5/17/83; Neal H. Cohen, M.D., a co–investigator will assume responsibility for this study.

10–06–86 Submitted a progress report.

10–31–86 Amended IND to provide for revisions to clinical study 20, "Evaluation of Epoprostenol Sodium and Subsequent Therapy in the Treatment of Primary Pulmonary Hypertension (PPH) in Adults," being conducted by Lewis Rubin, M.D. and Bertron Groves, M.D. The protocol for this study was submitted on June 24, 1982.

04–23–87. Amended IND to provide for clinical study 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," to be conducted by Robyn J. Barst, M.D

04–28–87 Telephone call to FDA informing them of the death of patient #101 who was enrolled in study 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D.

05–04–87 Submitted an adverse experience report on a patient (#101, PLC) who expired following treatment under clinical study 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D. Also submitted information on a patient who expired following treatment with FLOLAN during an attempted cardiopulmonary resuscitation.

05–07–87 Telephone call to FDA to clarify procedure for submission of adverse experience reports during NDA review; ADR's should be submitted to both the IND and NDA until NDA is approved.

06–18–87 Amended IND to provide for revisions to clinical study 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients." In addition, registered Lewis Rubin, M.D. as an investigator for study 36. The protocol for this study was submitted April 23, 1987.

07–01–87 Amended IND to provide for clinical study 35, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients," to be conducted by Lewis Rubin, M.D.

07–31–87 Submitted adverse experience report on a patient (#02, CJD), who experienced embolic cerebrovascular accident while being treated under protocol 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Lewis Rubin, M.D.

08–21–87 Telephone call from FDA regarding adverse experience (cerebrovascular accident) submitted on July 31, 1987. We were requested to notify investigators of this experience.

08–26–87 Amended IND to provide for clinical study 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," to be conducted by Lewis Rubin, M.D.

09–02–87 Letter from FDA confirming telephone conversation of August 21, 1987 and requesting that we notify investigators of adverse experience (cerebrovascular accident) submitted July 31, 1987.

09–04–87 Submitted a follow–up to our May 4, 1987 submission concerning the death of patient #101 (PLC) who was being treated under protocol 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III or IV Primary Pulmonary Hypertension."

09–11–87 Telephone call from FDA with concerns regarding protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," submitted on August 26, 1987.

09–15–87 Telephone call to FDA to review the status of patient entry and outcome for studies 35, 36 and 37.

09–25–87 Letter from FDA requesting that we submit additional safety report summaries at three month intervals for study 37–01, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension."

09–28–87 Submitted copy of our letter to investigators notifying them of adverse experience concerning patient #02, in response to FDA letter of September 2, 1987.

10–22–87 001 Amended IND to register Alfred Fishman, M.D. and Harold Palevsky, M.D. as investigators for the following clinical studies:

Protocol 35 – "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients," submitted on July 1, 1987.

Protocol 36 – "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," submitted on April 23, 1987 and amended on June 18, 1987.

10–30–87 002 Submitted a copy of letter to FDA that had been forwarded to all investigators concerning safety precautions, re: protocols 35, 36, and 37, as a follow–up to our September 28, 1987 submission.

11–09–87 003 Submitted three–month update covering period up to October 1, 1987 for the following clinical studies as requested by FDA in letter of September 25, 1987:

Protocol 35 – "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients"

Protocol 36 – "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients"

Protocol 37 – "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension"

11–16–87 004 — Amended IND to provide for clinical study 40, "Evaluation of the Hemodynamic Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," to be conducted by Matthew Horn, M.D., and Kenneth Moser, M.D.

12–21–87 005 — Amended IND to register Lewis Rubin, M.D. as an investigator for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure." Protocol for study was submitted on November 16, 1987 (004).

01–21–88 006 — Amended IND to register Mitchell Friedman, M.D. as an investigator for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987. Also registered Robyn Barst, M.D. as an investigator for clinical study 21, "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary Vasoreactivity in Patients with Primary Pulmonary Hypertension," submitted on July 27, 1982 and amended on May 17, 1983. Dr. Barst is replacing Thomas Starc, M.D.

01–29–88 007 — Submitted the second three–month safety update for studies 35, 36 and 37 covering the period from October 1, 1987 to December 31, 1987.

02–22–88 008 — Amended IND to register Michael D. McGoon, M.D. as an investigator for clinical study 35, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients," submitted on July 1, 1987 and clinical study 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," submitted on April 23, 1987 and amended on June 18, 1987. In addition, registered Alfred Fishman, M.D. and Harold Palevsky, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Dosease Patients with Acute Respiratory Failure," submitted on November 16, 1987.

03–08–88 009 — Submitted annual report.

03–25–88 010 — Amended IND to provide for revisions to clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004). The protocol is being conducted by the following investigators:

Lewis Rubin, M.D.	Alfred Fishman, M.D.
Harold Palevsky, M.D.	Kenneth Moser, M.D.
Mitchell Friedman, M.D.	Edgar Caldwell, M.D.
William Williams, M.D.	

	Also registered Michael McGoon, M.D. as an investigator for clinical study 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in patients with Primary Pulmonary Hypertension," submitted on August 26, 1987.
03–31–88	Telephone call to FDA to report the death of patient #17 who expired while being treated under clinical study 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D.
04–21–88 011	Submitted an adverse experience report on a patient (#18, JAF) who experienced flushing, faintness, nausea, hypotension and bradycardia while being treated under protocol 36, "Multicenter Evaluation of Long–Term FLOLAN in New York Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Lewis Rubin, M.D.
04–26–88 012	Submitted the third three–month safety update for the followingclinical studies: Protocol 35 – "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients" Protocol 36 – "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients" Protocol 37 – "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension"
04–27–88 013	Submitted summary information on patient #17 (VS) who expired while being treated under clinical study 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D.
05–05–88 014	Submitted an adverse experience report on patient #01 (REC) who experienced cough syncope and bronchitis while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
05–23–88 016	Amended IND to register Frederick Glauser, M.D., Paul Fairman, M.D. and Curt Sessler, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004).
06–27–88 017	Amended IND to register Keith Mansel, M.D. and James E. Griffith, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004).

07–08–88 018 Submitted an adverse experience report on patient #18 (JAF) who experienced syncope while being treated under protocol 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Lewis Rubin, M.D.

07–08–88 Telephone call to FDA informing them of patient #01 (REC) who experienced skin and eye photosensitivity while being treated under clinical study 37–01, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.

07–11–88 Telephone call to FDA informing them of patient #16 (DMC) who exprienced an increase in pulmonary arterial pressure while being treated under protocol 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Michael McGoon, M.D.

07–15–88 019 Submitted an adverse experience report on patient #01 (REC) who experienced skin and eye photosensitivity while being treated under protocol 37–01, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.

07–20–88 020 Submitted an adverse experience report on patient #16 (DMC) who experienced an increase in pulmonary arterial pressure (PAP) while enrolled in clinical study 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Michael McGoon, M.D.

07–27–88 021 As a follow–up to our telephone call of March 31, 1988, and letter of April 17, 1988, submitted an autopsy report on patient #17 (VS) who expired while being treated under protocol 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D.

07–28–88 022 Amended IND to register Ronald Pearl, M.D., Ph.D., Steve Jenkinson, M.D., Charles Bryan, M.D., Warren Summer, M.D. and Bennett deBoisblanc, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004).

08–10–88 023 Submitted annual report.

09–08–88 026	Submitted an adverse experience report on patient #06 (DMC)being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Michael McGoon, M.D.
09–13–88	Telephone call to FDA to explain our delay in supplying the last 90–day safety report for primary pulmonary hypertension.
09–14–88 028	Submitted the fourth three–month safety update for the following studies: Protocol 35 – Multicenter Evaluation of Long–Term FLOLAN Infusions inNew York Heart Asociation Clases I and II Primary Pulmonary Hypertension Patients Protocol 36 – Multicenter Evaluation of Long–Term FLOLAN Infusions inNew York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients Protocol 37 – Open Multicenter Evaluation of the Effects of ChronicFLOLAN Infusions in Patients with Primary Pulmonary Hypertension.
09–22–88 029	Amended IND to register Kenneth M. Moser, M.D. and KentKapitan, M.D. as investigators for clinical study 36, "Multicenter Evaluation on Long–Term FLOLAN Infusions in New York Heart ASsociation Classes III and IV Primary Pulmonary Patients," submitted on April 23, 1987 and amended on June 18, 1987. Also, registerd James Williams, M.D. and Kenneth Weir, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted November 16, 1987 (Serial No. 004).
10–20–88	Telephone call to FDA to report an adverse event in which patient #12 experienced pulmonary edema while being treated under protocol 36.
10–24–88 030	Amended IND to register Michael Nochomovitz, M.D. as an investigator for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004).
10–24–88	Telephone call to FDA to report the death of patient #04 being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
10–25–88 031	As a follow–up to the submission of September 8, 1988, submitted the hospital record for patient #06 (DMC) being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Michael McGoon, M.D.

10–25–88 032 — Amended IND to provide for revisions to clinical study 37, "OpenMulticenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D. and Michael McGoon, M.D. The protocol for this study was submitteed on August 26, 1987.

11–03–88 — Telephone call to FDA to report that patient #06 (DMC) experienced cramps, light–headedness, rapid pulse, lowered blood pressure and subsequent non–responsiveness while being treated under protocol 37, being conducted by Michael McGoon, M.D.

11–08–88 033 — Submitted an adverse experience report on patient #12 (MBS) whoexperienced severe, life–threatening pulmonary edema while enrolled under protocol 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Michael McGoon, M.D.

11–11–88 034 — Submitted an adverse experience report on patient #01 (WRS) who expired while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.

11–15–88 035 — Submitted an adverse experience report on patient #06 (DMC) who experienced altered mental status and hypotension while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Michael McGoon, M.D.

12–08–88 036 — Amended IND to provide for revisions to clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004).

12–30–88 037 — Amended IND to register Harold I. Palevsky, M.D. as an investigator for clinical study 21, "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Primary Vasoreactivity in Patients with Primary Pulmonary Hypertension and Selected Patients with Secondary Pulmonary Hypertension," submitted on July 27, 1982 and amended on May 19, 1983.

01–10–89 038 — Submitted the fifth three–month safety update for the following studies:

Protocol 35 – "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes I and II Primary Pulmonary Hypertension Patients."

Protocol 36 – "Multicenter Evaluaiton of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients."

Protocol 37 – "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension."

01–20–89 039 Amended IND to register Frank Lewis, M.D. as an investigator for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004) and amended on December 8, 1988 (Serial No. 036).

03–13–89 Telephone call to FDA to report an adverse event experience by patient #6 who became cyanotic, collapsed and developed a seizure while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension," being conducted by Michael McGoon, M.D.

03–17–89 040 In accord with FDA letter of September 25, 1987, submitted the sixth three–month safety update which covers the period from October 1, 1988 to December 31, 1988 for the following studies:

Protocol 36 – "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients"

Protocol 37 – "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension

03–29–89 041 Amended IND to register Stephen Jenkinson, M.D. and Charles Bryan, M.D. as investigators for clinical study 40, "Evaluation of the Hemodynamic and Oxygen Transport Effects of FLOLAN in Chronic Obstructive Pulmonary Disease Patients with Acute Respiratory Failure," submitted on November 16, 1987 (Serial No. 004) and amended on December 8, 1988 (Serial No. 036).

03–30–89 042 Submitted an adverse experience report on patient #6 (DMC) who experienced cyanosis, absence of respirations, seizure activity and loss of consciousness while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension," being conducted by Michael McGoon, M.D.

04–26–89 Telephone call to FDA to report that patient #05, enrolled in study 37, had an AutoSyringe pump malfunction, and developed SOB and severe dyspnea.

05–11–89 043 Submitted an adverse experience report on patient #05 (JAF) who experienced a sudden onset of dyspnea while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.

05–31–89 044 Submitted the seventh three–month safety update for the following studies:

Protocol 36 – "Multicenter Evaluation of Long–Term FLOLAN Infusions inNew York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients

	Protocol 37 – "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension"
07–11–89	Telephone conversation with FDA in which we were informed that the 3–month safety updates for FLOLAN in treating patients with primary pulmonary hypertension could be discontinued.
07–26–89 045	Letter to FDA confirming telephone agreement that 3–month safety updates for protocols 35, 36 and 37 would be discontinued.
08–10–89 046	Submitted annual report.
09–08–89 047	Submitted an IND Safety Report on patient #24 (DPM) who expired while being treated under protocol 21, 11 Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Primary Vasoreactivity in Patients with Primary Pulmonary Hypertension and Selected Patients with Secondary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
09–25–89	Letter to FDA notifying them of additional patient recruitment (3) in Protocol 36.
11–07–89	Telephone call to FDA to inform them that patient #28 expired while being treated under protocol 36 being conducted by Robyn Barst, M.D.
11–28–89	Telephone call to FDA to inform them of the death of patient #9 who was being treated under protocol 36 being conducted by Lewis Rubin, M.D.
12–13–89 049	Submitted IND Safety Report on patient #28 (AB) who expired while being treated under protocol 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classes III and IV Primary Pulmonary Hypertension Patients," being conducted by Robyn Barst, M.D.
01–22–90 050	Submitted IND Safety Report on patient #09 (JAW) who expired while enrolled in the control group of clinical study 36, "Multicenter Evaluation of Long–Term FLOLAN Infusions in New York Heart Association Classses III and IV Primary Pulmonary Hypertension Patients," being conducted by lewis Rubin, M.D.
02–19–90 052	Amended IND to register Robyn J.Barst, M.D. as an investigator for clinical study 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," submitted on August 26, 1987 and amended on October 25, 1988 (032).
02–20–90	Telephone call to FDA to report the death of patient #2 (PPH) who expired while being treated under protocol 37.
03–06–90	Telephone call to FDA to inform them of the publication of an article describing FLOLAN use in Primary Pulmonary Hypertension which is scheduled to appear in the April issue of Annals of Internal Medicine and to discuss the results of a television interview on CNN with one of our investigators and one of the patients receiving FLOLAN for PPH.

03–14–90	Submitted data to FDA in preparation for a meeting tentatively scheduled for April 4, 1990 to discuss recent results describing the use of FLOLAN in treating patients with Primary Pulmonary Hypertensin and the implications of these results on the possible submission of a Treatment IND.
03–29–90 054	In reference to our submission of March 14, 1990 which provided background material for the meeting of April 4, 1990, submitted a tentative list of Burroughs Wellcome attendees for that meeting and a reprint of an article from the 1987 British Heart Journal detailing FLOLAN's use in Primary Pulmonary Hypertension.
03–29–90 055	Submitted a written report and a report on the stoppage of the pump concerning patient #2 (CJD) who expired while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
03–30–90	Telephone call to FDA to inform them of the death of patient #36 (RMD) being treated under protocol 21 by Dr. Lewis Rubin.
04–05–90 056	Letter to FDA authorizing them to refer to our IND 16,459 and to our pending NDA 19–607 on behalf of the Upjohn Company, Kalamazoo, Michigan.
04–19–90	Telephone call from FDA to discuss FLOLAN's development as a diagnostic agent in primary pulmonary hypertension.
04–25–90 057	Submitted an IND Safety Report on patient #36 (RMD) who experienced cardiopulmonary arrest and subsequently expired while being treated under protocol 21, "Multicenter Emergency Evaluation of Epoprostenol Sodium in the Assessment of Pulmonary and Vasoreactivity in Patients with Primary Pulmonary Hypertension (PPH)" being conducted by Lewis Rubin, M.D.
05–09–90	Letter from FDA with their summary of the April 4, 1990, meeting.
05–23–90 058	Submitted a report on patient #01 (REC) who experienced catheter sepsis, bacteremia, and microscopic hematuria while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hy[ertension," being conducted by Lewis Rubin, M.D.
05–23–90 059	Submitted a report on patient #15 (RV) who experienced infection at catheter site while being treated under protocol 37, ""Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hy[ertension," being conducted by Robyn Barst, M.D.
06–12–90	Telephone call to FDA to inform them of the death of patient #08 (HLC) who suffered a cardiac arrest and subsequently expired while being treated under protocol 37.

06–28–90 060	In reference to meeting held on April 4, 1990, to discuss the use of FLOLAN in treating patients with Primary Pulmonary Hypertension, submitted our minutes of that meeting and comments regarding the FDA minutes of that meeting, together with a copy of the videotape of the CNN broadcast concerning FLOLAN.
06–28–90	Submitted our minutes of the April 4, 1990, meeting and comments regarding the FDA minutes of that meeting.
07–02–90 061	Submitted an IND Safety Report on patient #08 (HLC) who suffered acardiac arrest and subsequently expired while being treated under protocol 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusion in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
07–10–90	In reference to our Letter to the FDA of June 28, 1990 regarding the material on the videotape of the CNN broadcast, letter from FDA requesting a detailed, up–to–date report on the patient presented on that broadcast.
08–02–90 062	Submitted the case history for the patient presented on the videotape of the CNN broadcast as requested in the FDA letter of July 10, 1990.
09–05–90	Memo to FDA detailing three separate datasets utilized in these analyses: NIH NHLBI PPH National Registry Data; Papworth Hospital (UK) Data; and BW FLOLAN Protocols 35, 36, 37 Data.
09–18–90 063	Submitted data which provides evidence that FLOLAN is safe and effective therapy that prolongs survival and improves exercise capacity in NYHA Class III and IV patients with PPH; requested meeting to discuss possible Treatment IND.
10–05–90 064	In reference to telephone conversation of October 4, 1990, submitted summary information on Patient #16, (JJD) who experienced symptoms probably due to infusion pump failure while being treated under protocol 37, "O(pen Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension," being conducted by Lewis Rubin, M.D.
10–26–90	Telephone call to FDA regarding the review status of our September 18, 1990 submission that was in support of a possible treatment IND and to discuss the Subpart E status for this drug.
10–26–90 thru 01–10–91	Telephone contacts with FDA regarding the status of their review of our September 18, 1990 submission in support of the Treatment IND.
10–31–90	Telephone call from FDA to inform us that Subpart E status for FLOLAN would be granted and their review of our submission regarding the filing of a Treatment IND had not been completed.
11–05–90	In response to our request of June 28, 1990, letter from FDA informing us that FLOLAN qualifies for the special procedures designed to expedite the development, evaluation, and marketing of new therapies as delineated in Subpart E.

11–09–90 065	Amended IND to provide for revisions in the manufacturing and controls data for the use in clinical trials with FLOLAN Sterile Powder.
11–13–90	Letter from FDA requesting a progress report.
12–20–90 067	Submitted IND Safety Reports on the following patients being treated under protocol 37 by Lewis Rubin, M.D.: Patient #16 (JJD) who experienced shortness of breath, blackout, tightness in chest and diarrhea (9/3/90); and weakness, clamminess, faintness, shortness of breath, diarrhea, pallor, inability to move and glazed eyes (9/26/90); and patient #1 (REC) who experienced hypotension and grand mal seizure (following inadvertent overdose of FLOLAN).
12–21–90 068	Submitted IND Safety Reports on the following patients being treated under protocol 37 by Lewis Rubin, M.D.: Patient #07 (ECT) who experienced the loss of short term memory; andpatient #13 (LLW) who experienced a symptom complex which included the inability to move, lightheadedness, anxiety, left arm pain, diarrhea, pallor, clamminess, diaphoresis and tachycardia.
01–14–91	Conference call with FDA to discuss their proposal that we file a Treatment IND and follow the mortality in additional patients under the Treatment IND.
01–17–91 070	Submitted an Adverse Experience Report on Patient #07 (ECT) who experienced shortness of breath/dyspnea, diarrhea, blackout/disorientation, vomiting and lightheadedness while being treated under protocol 37 (conducted by Lewis Rubin.)
01–18–91 071	Submitted Adverse Experience Reports on the following patients being treated under protocol 37: Patient #17 (KP) who experienced sepsis while being treated by Robyn Barst, M.D.; and patient #01 (REC) who experienced a serious thrombosis near or in the indwelling catheter and sepsis while being treated by Lewis Rubin, M.D.
01–22–91 072	Submitted Annual Report which covers the period of April 1, 1989 through March 31, 1990.
01–22–91	As a follow–up to conference call with FDA on January 14, 1991, telephone call to FDA to obtain proposed dates they could meet with us to discuss further the Treatment IND.
01–23–91	In response to our January 22, 1991 call regarding possible meeting dates to discuss the treatment IND, telephone call from FDA requesting that we submit a written request for a meeting, a list of the BW Co. attendees and any new information we intend to present to them.
01–28–91	Telephone call to FDA informing them that we would be submitting a written request for a meeting and a list of meeting attendees as requested by the FDA on January 23, 1991.
01–30–91	Telephone call from FDA informing us that the meeting date to discuss the Treatment IND was scheduled on February 14, 1991

01–31–91	Telephone call to FDA to confirm that Feburary 14, 1991 would be a suitable date for the meeting.
02–06–91	Letter to FDA confirming the meeting scheduled for February 14, 1991 and submitting 1) a list of attendees; 2) a proposed agenda for the meeting; and 3) Summary of Updated Survival Data in preparation for the meeting.
02–06–91 074	Letter to FDA confirming the meeting scheduled for February 14, 1991.
02–13–91	Letter from FDA requesting a progress report.
02–18–91 073	Submitted an Adverse Experience Report on patient #17 (KP) who experienced sepsis, hypotension, low cardiac output and nausea while enrolled under protocol #17, being conducted by Robyn Barst, M.D.
04–03–91 079	Submitted a copy of our minutes of FDA meeting held on February 14, 1991, regarding the use of FLOLAN in the treatment of PPH.
04–04–91	Telephone conversation with FDA to clarify our position on a request received by the FDA from a Dr. Prince for emergency treatment of a patient (KW) with PPH.
07–12–91 090	Amended IND to provide for an additional 1.5 mg strength of FLOLAN Sterile Powder.
07–31–91	As a follow–up to meeting of February 14, 1991, letter to FDA requesting a meeting to discuss our future development plans for FLOLAN in the treatment of primary pulmonary hypertension patients; provided summary data in preparation for the meeting.
08–16–91	Letter from FDA enclosing their summary of the meeting held on February 14, 1991.
19 Aug 91	Minutes of meeting with FDA to discuss 1) toxicology requirements for chronic use of FLOLAN in patients with PPH and CHF, 2) the proposed protocol for a second exercise tolerance study to support NDA approval for PPH, and 3) labeling specifications for the intravenous delivery system.
09–06–91 097	In reference to FDA letter of August 16, 1991, summarizing the February 14, 1991 meeting, letter to FDA to requesting clarification of the agency's position on the inclusion of mortality claims in the label if the drug is approved for PPH on the basis of a second exercise study.
09–11–91 099	In reference to meeting with FDA on August 19, 1991 submitted a revised protocol 46, "Multicenter, Open–Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy Alone in Patient with Severe Primary Pulmonary Hypertension," to be conducted by Robyn Barst, M.D. The protocol reflects the following changes: 1) randomization assignment will be stratified by center, NYHA Class and vasodilator use at baseline; and 2) conventional therapy medications will be held constant unless clinical necessity dictates otherwise.

10–04–91 101	Amended IND to provide for revisions to clinical study 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension". The protocol is being amended to allow use of a larger vial of FLOLAN Powder, use of an in–line filter, storage of vials at room temperature and preparation of higher concentrations of FLOLAN solutions.
10–29–91 103	Amended IND to register Robert Bourge, M.D. and Lewis Rubin, M.D. as an investigator to conduct clinical study 46, "A Multicenter, Open–Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional therapy Alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve–Week Study".
11–07–91	In response to our letter of September 6, 1991 requesting clarification of the agency's position on the inclusion of mortality claims in the label, letter from FDA stating that they would not rule out some mention of mortality results in the labeling if the drug is approved, but, mortality would not be promotable as an efficacy claim.
11–11–91 105	In reference to telephone conversation of September 23, 1991, as requested, submitted additional details on the randomization procedure to be used in the FLOLAN exercise trial in patients with PPH.
11–12–91 104	Submitted Annual Report which covers the period of April 1, 1990 thru March 31, 1991.
20 Nov 91	Submitted to FDA an Information Package in preparation for the End of Phase II meeting scheduled for 12 Dec 91.
12–05–91 106	Amended IND to provide for clinical study 47, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Continuation Study," to be conducted by Robyn Barst, M.D.
12–09–91	Letter to FDA to confirm the meeting scheduled for December 12 and submitted copies of our proposed agenda, a list of persons attending and a summary of the changes made in the draft clinical protocol previously provided to FDA.
12–13–91 107	Amended IND to register Neil Ettinger, M.D., Victor Tapson, M.D., Anthony Killian, M.D., Ph.D. and Stuart Rich, M.D. as investigators to conduct clinical study 46, "A Multicenter, Open–Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy Alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve–Week Study."

12–31–91
108 Amended IND to register David Badesch, M.D., FACP, FCCP, Bertron Groves, M.D. and Edgar Caldwell, M.D. to conduct clinical study 46, "A Multicenter, Open–Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy Alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve–Week Study" and Lewis Rubin, M.D., to conduct clinical study 47, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Continuation Study."

01–27–92
109 Amended IND to register William Clarke, M.D. as an investigator for clinical study 46, "A Multicenter, Open–Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy Alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve–Week Study" and Robert Bourge, M.D. for clinical study 47, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional therapy in Patients with Severe Primary Pulmonary Hypertension: A Continuation Study".

02–27–92
110 Amended IND to register the following investigators to conduct clinical study 46, "" Multicenter, Open–Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve–Week Study"

Bruce Brundage, M.D. Barry Uretsky, M.D.
Michael McGoon, M.D. Warren Summer, M.D.
Srinivas Murali, M.D., FACC, FACP

5 Mar 92 Telephone call to FDA regarding the death of patient #15999 (MVP) who experienced a pneumothorax secondary to an attempted Schwann–Ganz catheterization and subsequently expired while being treated under protocol 46 by Dr. Michael McGoon.

03–17–92
111 Amended IND to register David Langleben, M.D., Montreal, Canada as an investigator for clinical study 46, "A Multicenter, Open–Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve–Week Study".

03–31–92
112 In reference to telephone conversation of March 5, 1992, submitted IND Safety Report on patient #15999 (MVP) who experienced a pneumothorax secondary to an attempted Schwann–Ganz catheterization and subsequently expired while being treated under protocol 46.

04–01–92 113	Amended IND to register Spencer Koerner, M.D., and Nicholas Hill, M.D. as investigators to conduct clinical study 46, "A Multicenter, Open–Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve–Week Study" and Victor Tapson, M.D., David Badesch, M.D., Bertron Groves, M.D. and Stuart Rich, M.D. as investigators to conduct clinical study 47, "A Multicenter, Open–Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A continuation Study".
04–21–92 114	Amended IND to register Cesar Keller, M.D. as a investigator to conduct clinical study 46, "A Multicenter, Open–Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve–Week Study" and William Clarke, M.D. as a investigator to conduct clinical study 47, "A Multicenter, Open–Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A continuation Study".
20 May 92	Telephone call from FDA indicating that they would like a new NDA submitted for treatment of PPH and that we should resubmit all data rather than cross reference the hemodialysis NDA.
06–04–92 115	Amended Dr. Nicholas Hill's Form FDA 1572 to include James Klinger, M.D. as a subinvestigator to conduct clinical study 46, "A Multicenter, Open–Label, Randomized, Parallel Comparison of the Safety and Efficacy of Chronic FLOLAN Infusions Plus Conventional Therapy to Conventional Therapy alone in Patients with Severe Primary Pulmonary Hypertension: A Twelve–Week Study" and to register Bruce Brundage, M.D., Edgar Caldwell, M.D., Nicholas Hill, M.D., Srinivas Murali, M.D., and Barry Uretsky, M.D. as investigators to conduct clinical study 47, "A Multicenter, Open–Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Continuation Study".
06–11–92 116	In reference to meeting with FDA on August 19, 1991 and telephone conversation on May 22, 1992, submitted the following genetic toxicology study reports: Evaluation of U–53,217A in the Salmonella/Microsome Test (Ames Assay) (Doc. 7200/81/7263/001). The Micronucleus Test with Prostacyclin (U–53,217A) (Doc. 0013/81/7263/002). Evaluation of U–53,217A (PGI_2) in the DNA Damage/Alkaline Elution Assay (Doc. 7263/80/7263/023).

07–28–92 117	Amended IND to register Edgar Caldwell, M.D. as an investigator to conduct clinical study 37, "Open Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension" and Spencer Koerner, M.D., Cesar Keller, M.D., Michael McGoon, M.D. and Neil Ettinger, M.D. as investigators to conduct clinical study 47, "A Multicenter, Open–Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Continuation Study."
11 Aug 92	Telephone call from FDA, in response to our 10 Aug 92 telephone call, advising that summary data regarding preliminary survival results of our exercise tolerance study 46 be submitted for their review prior to any telephone discussion.
08–14–92 118	In reference to telephone conversation with FDA on August 11, 1992, submitted preliminary survival and exercise tolerance data which had recently become available from protocol 46, "A Multicenter, Open–Label, Randomized, Parallel, Controlled Comparison of FLOLAN plus Conventional Therapy to Conventional Therapy Alone in Patients with Severe, Primary Pulmonary Hypertension".
10 Aug 92	Telephone call to FDA informing them that our exercise tolerance study 46 was nearing completion and that preliminary survival results would be available by 13 Aug 92 and requesting their review of this data via telephone.
08–14–92	Letter from FDA to solicit our cooperation in establishing a single database containing ambulatory blood pressure measurements obtained from hypertensive patients receiving placebo in randomized clinical trials.
08–20–92 119	Amended IND to provide for clinical study 49, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Safety Study". Submitted an amended Form FDA 1572 for Dr. Neil Ettinger (previously submitted December 13, 1991 for protocol 46) who will be conducting this study. Study sites for this study will be limited to those investigators previously registered for protocol 46.
09–22–92 121	Amended IND to register Bennett deBoisBlanc, M.D. as an investigator for clinical study 47 and Victor Tapson, M.D. as an investigator for clinical study 49.
09–25–92 120	Amended IND to provide for the use of a single vial formulation of FLOLAN Injection, 0.5 mg and 1.5 mg, in clinical trials.
09–25–92 122	Letter to FDA to confirm the meeting to be held on October 6, 1992. As requested, submitted a complete statistical package for protocol 46, additional background information and an agenda for the meeting.
10–01–92	Telephone call to FDA to discuss an upcoming FLOLAN meeting.

6 Oct 92	Minutes of meeting with FDA to discuss 1) the clinical results from protocol 46, 2) the update on patients that were enrolled in protocol 45 and 47, 3) and their comments and suggestions regarding the TIND.
10–19–92	Letter from FDA requesting an Annual progress report.
10–20–92 123	Amended IND to register David Langleben, M.D., Montreal, Quebec, Canada as an investigator to conduct clinical study 47.
10–26–92 and 10–29–92	Telephone conversations with FDA to request their participation in BW's Advisory Committee for the FLOLAN TIND; it was the ir opinion that it would be more appropriate if they just reviewed documents as they were provided under the IND.
10–29–92 124	Amended IND to register Syed Jafri,M.D. (who is replacing Mihai Gheorghiade, M.D.) as an investigator to conduct clinical study 45 and to register the following investigators to conduct clinical study 49: David Badesch, M.D., FACP, FCCP Bertron Groves, M.D. William Clarke, M.D. Stuart Rich, M.D. Bruce Brundage, M.D. Lewis Rubin, M.D. Robyn Barst, M.D.
11–10–92 125	Amended IND to register Dr. David Langleben, Montreal, Quebec, Canada as an investigator to conduct clinical study 49.
11–20–92 126	As agreed in meeting with FDA on October 6, 1992, submitted a draft of the features required for the pump which will be used for chronic infusion of FLOLAN under our treatment IND.
11–25–92	Telephone call to FDA to report the death of patient #01103 who was being treated with FLOLAN under protocol 49; cause of death uncertain at this time.
12–09–92	Telephone call from FDA to discuss our pump specifications to be used for treatment of patients with PPH.
12–11–92	Telephone approval given to FDA authorizing them to refer to our IND on behalf of Robyn Barst, M.D., of New York, NY to support her IND41,252 for the treatment of a patient with pulmonary hypertension.
12–15–92 127	Amended protocol 47, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Continuation Study", to provide for the use of the single vial formulation which is supported by our Chemistry, Manufacturing, and Control Amendment submitted September 25, 1992.
12–30–92 128	As agreed in the meeting of October 6, 1992, submitted to FDA for review a draft copy of the protocol for our planned Treatment IND for FLOLAN.

01–07–93 129	Amended IND to register Adaani Frost, M.D., who is replacing Cesar Keller, M.D. as principal investigator, to conduct clinical study 47 and Robert Bourge, M.D., Edgar Caldwell, M.D. and Adaani Frost, M.D. as investigators to conduct clinical study 49.
01–11–93	Telephone call to FDA regarding the status of their review of the TIND protocol.
01–12–93 130	Letter to FDA authorizing them to refer to our IND on behalf of Robyn Barst, M.D., of New York, NY to support her IND 41,252 for the treatment of a patient with pulmonary hypertension.
01–13–93	Telephone call to FDA with questions regarding the upcoming PPH NDA.
01–14–93 131	Amended the following protocols to provide for the use of the single vial formulation: 37, "A Multicenter Evaluation of the Effects of Chronic FLOLAN Infusions in Patients with Primary Pulmonary Hypertension" and 49,"A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Safety Study".
01–15–93 132	Submitted to FDA copies of instructional videos ("Patient Information" and "The Clean Routine") and of same transcripts which will be used in educating patients entered into the Treatment IND protocol.
01–26–93	Telephone call to FDA to determine the status of their review of the proposed TIND protocol.
01–28–93	Telephone call from FDA to confirm that their review of the proposed TIND protocol would be completed by January 29, 1993.
01–28–93	Telephone call to FDA to discuss the adverse events reported by patients receiving the new single vial FLOLAN product.
02–01–93	Telephone call to FDA to follow–up on the review process of the TIND protocol.
02–04–93	Telephone call to FDA to provide an updated status report on the adverse experiences reported by eight of the patients receiving the new single vial formulation.
02–11–93 133	Amended IND to register Christopher McGregor, M.B., Ch.B., FRCS as an investigator to conduct clinical study 21 and to register Bennett de Boisblanc, M.D. and Srinivas Murali, M.D., FACC, FACP/Barry Uretsky, M.D. (Co–principal investigators) as investigators to conduct clinical study 49.
02–16–93	Panafax received from FDA with comments on CMC submission of September 25, 1992 providing for single vial formulation of FLOLAN.

02–18–93 134	Amended IND to provide for revisions (to allow recording of hemodynamic measurements and vital signs for analysis of the efficacy and safety of the FLOLAN reformulation) to clinical study 49, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusion Plus Conventional Therapy in Patients with Severe Primary Pulmonary Hypertension: A Safety Study".
02–18–93	Letter received from FDA advising that the proposed treatment IND protocol is acceptable but that the study may not be initiated until a satisfactory response to chemistry issues raised in their February 16, 1993 letter relevant to safety is submitted. are resolved. A copy of this letter was panafaxed to us on February 17, 1993.
03–05–93 135	In reference to telephone conversations with FDA on January 28 and February 4, 1993 and their letter of February 18, 1993, submitted to FDA a copy of our letter sent to investigators which summarized our findings documenting that the recent problems in some patients switched to the new formulation were not due to the new formulation, but were due to a variety of other factors which are preventable.
03–18–93 136	Amended IND to register Michael McGoon, M.D., Nicholas Hill, M.D. and Spencer Koerner, M.D. as investigators to conduct clinical study 49.
04–05–93	Letter from FDA requesting an IND annual report.
04–16–93 137	In reference to FDA's request of April 5, 1993, submitted an Annual Report for the period of April 1, 1991 through March 31, 1992 and submitted the revised Investigator's Brochure. Informed FDA of our intention in the future to submit INDs 16,459 and 38,609 as a combined IND Annual Report.
16 Apr 93	Telephone call to FDA to report the death of patient #02104 who was being treated under protocol 49 by Lewis Rubin, M.D.
10 Jun 93 138	Amended IND to register Cesar Keller, M.D. as an investigator to conduct clinical study 49.
17 Jun 93 139	Letter to FDA in reference to telephone conversation of 16 Apr 93, submitted an IND Safety Report for patient #02104 who experienced ventricular tachycardia and expired while being treated under protocol 49, "A Multicenter, Open Evaluation of the Safety of Chronic FLOLAN Infusions Plus Conventional Therapy in Patients with Primary Pulmonary Hypertension: A Safety Study ", being conducted by Dr. Lewis Rubin.
18 Jun 93	Telephone call to FDA to inform them of the termination of CHF study 48 by the DSMB; we assured FDA that this would not effect our plans for the PPH NDA.
28 Jun 93	Telephone call from FDA requesting the emergency treatment of a patient with severe PPH secondary to Lupus erythematosus under an investigator IND.

29 Jun 93	Telephone call to FDA in response to their request of 28 Jun 93 regarding our willingness to provide FLOLAN to a patient with severe PPH secondary to Lupus erythematosus under an investigator IND.
7 Jul 93 140	Letter to FDA in response to their letter of 16 Feb 93 with questions concerning the chemistry, manufacturing and controls data for the new single vial formulation in our amendment dated 25 Sep 92. Notified FDA that development of the single vial formulation has been discontinued.
9 Jul 93 141	Letter to FDA amending the IND to provide for Greenville, NC as an additional manufacturing site of the Sterile Diluent for FLOLAN for use in clinical trials.
9 Jul 93	Telephone call to FDA to report that Patient #15105 experienced acute pulmonary congestion and Patient #01102 experienced temporary blurry vision associated with a headache while enrolled under protocol 49.
19 Jul 93 143	Amended IND to provide for clinical study 50, "A Multicenter, Open, Compassionate–Use Protocol to Provide Chronic FLOLAN Infusions Plus Conventional Therapy to Patients with Severe Primary Pulmonary Hypertension", to be conducted by Bertron Groves, M.D. and David Badesch, M.D.
20 Jul 93 144	Amended IND to register Henry Mizgala, M.D., FRCP, Vancouver, British Columbia, Canada as an investigator to conduct clinical study 49.
24 Aug 93 145	Amended IND to register Stuart Rich, M.D., Neil Ettinger, M.D. and Adaani Frost, M.D. as investigators to conduct clinical study 50.
27 Aug 93 146	Letter to FDA submitting an Annual Report for the period 1 Apr 92 through 31 March 93.
2 Sep 93 147	Amended IND to register Robert Bourge, M.D. as an investigator to conduct clinical study 21 and to register Spencer Koerner, M.D. as an investigator to conduct clinical study 50.
9 Sep 93 148	Letter to FDA, in reference to telephone conversation on 2 Sep 93, requesting a meeting with FDA during the week of 18 October 93 to discuss the NDA filing strategy and to address the manufacturing and controls data intended to support the filing.
9 Sep 93	Telephone call to FDA to request a meeting with FDA to discuss Chemistry, Manufacturing and Control issues regarding our planned NDA and to update them on our plans regarding the single vial formulation, NDA strategy and the Compassionate Use Trial v. Treatment IND.
23 Sep 93 149	Amended IND to register William Clarke, M.D. as an investigator to conduct clinical study 50.
23 Sep 93	Telephone call from FDA to advise that the meeting we requested on 9 Sep 93 to discuss CMC issues in connection with our proposed NDA has been scheduled for 15 Nov 93.

30 Sep 93 150	Amended IND to provide for revisions (amendment #1, to allow those patients in continuation protocols 37, 47, and 49 to be enrolled in protocol 50) to clinical study 50. .
8 Oct 93	Letter from The Upjohn Company along with a copy of their letter from FDA granting them approval to export epoprostenol sodium to Spain.
15 Oct 93 151	Amended IND to register Robyn Barst, M.D., Cesar Keller, M.D., and Edgar Caldwell, M.D. as investigators to conduct clinical study 50.
25 Oct 93	Telephone call to FDA to confirm the meeting scheduled for 15 Nov 93 and to inform them that background data for the meeting would be sent this week.
27 Oct 93 152	Amended IND to register Michael McGoon, M.D. as an investigator to conduct clinical study 50.
29 Oct 93 153	Letter to FDA, in reference to telephone conversations on 9 and 23 Sep 93 and 25 Oct 93 concerning our request for a meeting to discuss our upcoming NDA, submitting nine copies of summary information of the chemistry, manufacturing, and controls data for pre–meeting review by the attendees and a revised agenda for the 15 Nov 93 meeting.
19 Nov 93 154	Amended IND to register Bruce Brundage, M.D. and Lewis Rubin, M.D. as investigators to conduct clinical study 50.
23 Nov 93 155	Letter to FDA submitting minutes from the 15 Nov 93 meeting and a full copy of the information presented.
29 Nov 93 156	Letter to FDA submitting an IND Safety Report for patient #89 who developed severe hypotension and died after discontinuing FLOLAN while enrolled in clinical study 21, being conducted by Dr. Robyn Barst; also submitted a copy of the Dear Dr. letter addressing this experience.
10 Dec 93 157	Letter to FDA submitting stability data and data supporting the reconstitution and dilution studies to be evaluated for their acceptability for filing in the NDA as requested in the meeting held on 15 Nov 93.
12 Jan 94 158	Amended IND to register Nicholas Hill, M.D. and Robert Bourge, M.D. as investigators to conduct clinical study 50.
14 Jan 94 159	Letter to FDA, in reference to telephone conversation of 7 Jan 94, submitting an IND Safety Report for patient #09205, who experienced hypotension while enrolled in study 50, being conducted by Dr. Bruce Brundage. Also submitted a copy of the Dear Dr. letter pertaining to this incident.
14 Jan 94 160	Amended IND to provide for revisions (amendment #2) to clinical study 50.
1 Feb 94	Letter from FDA with requests regarding our 10 December 93 submission which provided stability data and data supporting the reconstitution and dilution studies.

9 Feb 94 161	Amended IND to register Srinivas Murali, M.D. and Barry Uretsky, M.D. as co–principal investigators to conduct clinical study 50.
17 Feb 94 162	Letter to FDA submitting our response to comments contained in their 1 February 1994 letter regarding our 10 December 93 submission.
28 Feb 94 163	Letter to FDA submitting the final report for an ongoing reconstitution study in response to FDA's comments in their 1 Feb 94 letter regarding our amendment of 10 Dec 93 providing stability data and data supporting the reconstitution and dilution studies. This data was also included in the NDA submitted on 28 Feb 94.
1 Mar 94 164	Amended IND to register Jay Fricker, M.D. as an investigator to conduct clinical study 50.
3 Mar 94 165	Letter to FDA submitting a "Summary of the Pharmaceutics, Clinical Pharmacology and Preclinical Pharmacology" on the single–vial formulation used in clinical trials for treating patients with PPH. This summary was incorporated by reference to NDA 20–444 as agreed in our meeting with FDA on 15 Nov 93.
31 Mar 94 166	Letter to FDA, in reference to telephone conversation of 22 Mar 94, submitting IND Safety Reports for two patients who were enrolled in protocol 50: Patient #17111 who experienced a cardiac arrest and subsequently expired while being treated by Dr. Adaani Frost; and Patient #01011 who experienced hemothorax and expired while being treated by Dr. Robyn Barst.
31 Mar 94 167	Amended IND to register David Langleben, M.D., Montreal, Quebec, Canada as an investigator to conduct clinical study 50.
6 Apr 94 168	Amended IND to register Bennett deBoisblanc, M.D. as an investigator to conduct clinical study 50.
11 Apr 94 169	Amended IND to register Victor Tapson, M.D. as an investigator to conduct clinical study 50.
11 Apr 94	Letter to FDA submitting an adverse event report involving the CADD–1 portable infusion pump as a follow–up to our IND Safety Report submitted on 31 Mar 94.
22 Apr 94	Telephone call from FDA to inform us of a request from Dr. Robert Baker to treat a patient with pulmonary hypertension.
26 Apr 94 170	Amended IND to register David Badesch, M.D. as a co–principal investigator with Bertron Groves, M.D. to conduct clinical study 21.
29 Apr 94 171	Letter to FDA, submitting an IND Safety Report for patient #16201 who experienced intrapulmonary hemorrhage and died while enrolled in protocol 50, being conducted by Dr. David Langleben.
3 May 94 172	Amended IND to register David Ostrow, M.D., Vancouver, British Columbia, Canada to conduct clinical study 50.

11 May 94 173	Letter to FDA, in reference to telephone conversation of 29 Apr 94, submitting an IND Safety Report for patient #01113 who died suddenly while enrolled in study 50, being conducted by Dr. Robyn Barst.
25 May 94	Telephone call from FDA regarding a Congressional inquiry concerning a request which BW had denied for treatment of a patient (CH) with PPH due to the potential risks to the patient being greater than the possible benefits. Two conference calls on this same date resulted in BW agreeing to confer with the investigator, Stuart Rich, M.D. following a further evaluation of the patient's condition.
1 Jun 94 174	Letter to FDA, in reference to telephone conversation on 24 May 94, submitting an IND Safety Report for patient #10202 who experienced a transient ischemic attack while enrolled in study 50, being conducted by Nicholas Hill, M.D. Also, as requested, submitted the pulmonary artery pressure results at catherization and a copy of the letter to investigators addressing this experience.
11 Jul 94 175	Letter to FDA submitting an Annual Report for the period 1 Apr 93 through 31 Mar 94.
5 Aug 94 176	Letter to FDA submitting an IND Safety Report for patient #04204 who experienced a migraine headache while enrolled in clinical study 50, being conducted by Edgar Caldwell, M.D.
1 Nov 94	Telephone call to FDA to discuss the Dr. Stuart Rich's (investigator) request to treat a female patient with PPH, who is pregnant, under protocol 50. FDA approved the treatment exception providing that an IRB approval and a signed informed consent form were required from the patient.
8 Nov 94	Telephone call to FDA to provide a summary on a patient who experienced bradycardia, syncope and died while being treated on a compassionate basis in Japan.
23 Nov 94 177	Letter to FDA, in reference to telephone conversation of 8 Nov 94, submitting an IND Safety Report for patient N–Y who experienced fatal bradycardia while being treated on a compassionate basis in Japan.
1 Dec 94 178	Letter to FDA submitting an IND Safety Report for patient #14201 who experienced post operative bleeding while enrolled in clinical study 50, being conducted by William Clarke, M.D.
17 Jan 95 179	Letter to FDA submitting an IND Safety Report for patient #17112 who experienced suspected anaphylaxis to streptokinase and subsequently died while enrolled in protocol 50, being conducted by Dr. Adaani Frost.
31 Mar 95 180	Letter to FDA, in reference to the IND Safety Report submitted on 17 Jan 95, submitting the provisional autopsy report for patient #17112 who experienced suspected anaphylaxis to streptokinase and subsequently died while enrolled in protocol 50.

11 Apr 95 181	Letter to FDA, in reference to telephone conversation of 4 Apr 95, submitting an IND Safety Report for patient #13205 who developed a hemopneumothorax during Hickman catheter replacement due to acinetobacter infection and subsequently died while enrolled under protocol 50, being conducted by Dr. Srinivas Murali. In addition, submitted an IND Safety Report for patient #17202 who developed a hemothorax during Hickman catheter replacement to alleviate recurrent line while enrolled under protocol 50, being conducted by Dr. Adaani Frost.
8 May 95 182	Letter to FDA submitting an IND Safety Report for patient #07201 who experienced bacteremia with related immune–complex glomerulonephritis while enrolled in protocol 50, being conducted by David Badesch, M.D.
10 May 95	Telephone call to FDA to inform of the death of patient #09244 who was being treated under protocol 50.
17 May 95 183	Letter to FDA submitting an IND Safety Report for patient #15211 who experienced pulmonary edema while enrolled in protocol 50, being conducted by Michael McGoon, M.D.
18 May 95 184	Letter to FDA submitting an IND Safety Report for patient #09244 who experienced hypotension and subsequently expired while enrolled in protocol 50, being conducted by Dr. Bruce Brundage.
16 Jun 95	Telephone call to FDA to report an adverse event for a patient enrolled under protocol 21.
19 Jun 95 185	Letter to FDA submitting an IND Safety Report for patient #07006 who bled intermittently from the catheter insertion site, over a three day period following Hickman catheter replacement, while enrolled in protocol 50, being conducted by Dr. David Badesch.
22 Jun 95	Telephone call to FDA to report that patient #02218 experienced endocarditis and subsequently died while being treated under protocol 050.
27 Jun 95 186	Letter to FDA in reference to telephone conversations of 16 and 22 Jun 95, submitting an IND Safety Report for patient #82 who experienced increased shunting with hypoxemia while enrolled in protocol 21, being conducted by Dr. Berton Groves and for patient #02218 who experienced endocarditis while enrolled in protocol 50, being conducted by Dr. Lewis Rubin.
28 Jun 95 187	Amended IND to register Brad Warner, M.D. as an investigator to conduct clinical study 21.
14 Aug 95 188	Letter to FDA, in reference to our 15 Oct 95 submission registering Dr. Robyn Barst of Columbia Presbyterian Babies Hospital for protocol 50, informing them of the addition of Quantum Health Resources, 150 Lake Dr., Wexford PA as a Contract Research Organization for distribution of CTM and related supplies to Columbia Presbyterian Babies Hospital.

25 Feb 94	Submitted user fee for this NDA.
28 Feb 94	Submitted an Original New Drug Application to provide for use in the treatment of primary pulmonary hypertension (PPH).
28 Feb 94	Submitted methods validation for the original NDA.
2 Mar 94	Telephone conversation with FDA to discuss the biopharmaceutics/pharmacokinetics reviewer's request for stereospecific pharmacokinetic information.
8 Mar 94	Letter from FDA acknowledging receipt of our NDA submitted; if accepted, 28 February 1994; stated that 29 April 1994 will be the filing date.
11 Mar 94	Submitted a User Fee Cover Sheet to complete User Fee requirements for our primary pulmonary hypertension NDA submitted 28 February 1994.
30 Mar 94	Telephone call from FDA to request diskettes containing the SAS listings for pivotal studies 35/36 and 46.
31 Mar 94	Telephone call from FDA to request 1) Normal Values for pulmonary hemodynamics from two hospitals used in the pivotal trials (studies 35/36/46); 2) a definition of Wood Units as a measure of vascular resistance; 3) a clear description of the randomization procedure used in Study 46.
6 Apr 94	As requested by FDA in 30 March 1994 telephone conversation, submitted diskettes containing SAS listings for studies 35/36 and 46 along with hard copies of the annotated case report for each study and the phase/phase sequence/subset list.
7 Apr 94	In response to FDA telephone requests on 31 March 1994, submitted, 1) normal values for pulmonary hemodynamics from two hospitals used in the pivotal trials (studies 35/36/46); 2) a definition of Wood Units as a measure of vascular resistance; 3) a clear description of the randomization procedure used in Study 46.
8 Apr 94	Telephone call from FDA to request a meeting on 14 April 1994, to discuss FDA concerns, re: the Human Pharmacokinetics, Chemistry and Microbiology sections of our NDA.
14 Apr 94	Minutes of meeting with FDA to discuss NDA filing issues.
15 Apr 94	Telephone call to FDA to provide information regarding patients in Study 46 who received "Dartford" manufactured drug vx. "Upjohn" manufactured drug.
20 Apr 94	As requested by FDA in 14 April 1994 meeting, submitted "A Summary of the Use of Epoprostenol Sodium Synthesized by Wellcome Research Laboratories (Dartford, England) in Study 46" (Document No. THZZ/94/0185).
21 Apr 94	Received by panafax from FDA, a request for additional information needed for the NDA microbiology review.
22 Apr 94	Telephone call from FDA to request 1) a copy of the "Adaptive Randomization Computer Program" used for Study 46, and 2) patient identifiers used for the randomization code.

22 Apr 94	As requested in meeting with FDA on 14 April 1994, submitted "Rationale for Waiver of Human Pharmacokinetics and Bioavailability Requirements for FLOLAN (epoprostenol sodium) for Injection".
25 Apr 94	As requested by FDA in 22 April 1994 telephone call, submitted a copy of the "Adaptive Randomization Computer Program" used for the randomization procedure in Study 46; a copy of this randomization procedure was previously submitted on 11 November 1991 to our IND 16,459.
29 Apr 94	Submitted a summary of a telephone conference call with FDA on 20 April 1994, concerning our proposed stability data package and the microbiological review of our NDA.
29 Apr 94	Submitted responses to microbiology questions contained in the FDA letter received by telefacsimile on 21 February 1994; a field copy was also provided to the local FDA district office.
29 Apr 94	Telephone call to FDA in which the FDA confirmed that the NDA was accepted for filing this date.
4 May 94	Telephone call from FDA to request 1) an electronic and hard copy of the actual randomization program, 2) procedure for implementation, and 3) randomization code.
6 May 94	Letter from FDA with recommendations and requests concerning the environmental assessment submitted 28 February 1994.
6 May 94	As requested by FDA in 4 May 1994 telephone conversation, submitted diskettes containing the source code and the compiled code for the randomization program, and provided documentation describing the randomization procedure.
9 May 94	Letter from FDA requesting clarification on points relating to the NDA statistical review.
16 May 94	Telephone call from FDA's Compliance Division to request information to use in preparing for the clinical inspections in connection with our NDA.
17 May 94	Telephone call from FDA's Compliance Division requesting additional information for clinical inspections.
17 May 94	As requested in 16 May 1994 telephone conversation, panafaxed to FDA tables which provide a list of investigators for NDA studies 35, 36 and 46.
14 Apr 94	Minutes of meeting with FDA concerning the NDA Manufacturing and Control, including Microbiology (Item 3), and Human Pharmacokinetics (Item 6) data.
27 May 94	Submitted response to FDA letter of 9 May 1994, concerning the NDA statistical review.
27 May 94	As requested by FDA in 16 and 17 May 1994 telephone conversations, submitted information for FDA's use in preparing for clinical inspections.
2 Jun 94	Telephone call to FDA to obtain clarification to questions 1.c and 3.e, in the 6 May 1994 environmental assessment deficiency letter.

7 Jul 94	Letter to FDA providing authorization from The Upjohn Company for B.W. Co. to reference their Drug Master File No. 5319.
27 Jul 94	Submitted the four month safety update.
28 Jul 94	As requested by FDA in 26 July 1994 telephone call, submitted case report forms for patients in Study 46 who died or were transplanted.
1 Aug 94	Letter from FDA questions and requests concerning the NDA review.
3 Aug 94	Telephone call from FDA to request data tabulations of hemodynamic values collected in Study 46 for each study site and all patients.
5 Aug 94	As requested by FDA in 3 August 1994 telephone call, submitted data tabulations of hemodynamic values for each study site and all patients in study 46.
23 Aug 94	Submitted responses to FDA questions in their 1 August 1994 letter, re: NDA review.
14 Sep 94	Submitted a revised Environmental Assessment and our response to FDA comments contained in 6 May 1994 letter.
16 Sep 94	Submitted updated stability data as committed to 20 April 1994 telephone conference with FDA and our 29 April 1994 submission.
16 Sep 94	Telephone call from FDA's Compliance Division to request, after discussions with the Medical Officer, additional analysis of adverse drug reactions which occurred during the dose–ranging segment of Study 46.
23 Sep 94	Letter from FDA requesting information pertinent to the NDA clinical and statistical review.
23 Sep 94	Letter from FDA stating that our 14 September 1994 Environmental Assessment submission is considered a major amendment and that 60 additional days will be required to complete the NDA review.
23 Sep 94	Telephone call from FDA to review the status of our responses to their requests, re: stability update, and the Upjohn response to Drug Master File deficiencies.
28 Sep 94	Telephone call to FDA to obtain clarification of their requests in their 23 September 1994 letter, re: clinical and statistical information pertinent to the NDA review.
3 Oct 94	Telephone call from FDA to provide comments concerning the revised Environmental Assessment submitted 14 September 1994.
4 Oct 94	As requested by FDA in 16 September 1994 telephone conversation, submitted additional analyses of adverse drug reactions which occurred in the dose–ranging segment of study 46.

6 Oct 94	As requested by FDA in 3 October 1994 telephone conversation, provided replacement pages 1–4 and 7 of the Environmental Assessment submitted 14 September 1994.
17 Oct 94	Received by panafax from FDA, letter requesting additional or clarifying information to support the sterilization process validation information portion of our NDA.
20 Oct 94	Telephone call from FDA to advise that Flolan had been placed on the agenda for the 23–24 February 1995 Cardio–Renal Advisory Committee.
27 Oct 94	Letter to FDA requesting a meeting to discuss the Flolan Advisory Committee meeting scheduled for 23–24 February 1995.
28 Oct 94	As requested by FDA in 23 September 1994 telephone call, submitted additional clinical and statistical information for the NDA review.
1 Nov 94	Letter from FDA stating that the additional NDA clinical and statistical information submitted 28 October 1994, is considered a major amendment and the regulatory due date has been extended to 25 March 1995; the due date under the Prescription User Fee Act of 1992 remains 27 February 1995 .
10 Nov 94	Letter from FDA stating that the medical review of our NDA is complete and contact will be made to arrange a meeting to discuss the application and the Advisory Committee meeting which is scheduled for February. (Copies of medical and statistical reviews attached.)
10 Nov 94	Telephone call from FDA to report that BW Co. will be receiving a letter with review comments on the clinical section of the NDA, and they wish to schedule a meeting to discuss the comments.
18 Nov 94	Telephone conversation with FDA to discuss the NDA review; agreed that a meeting between BW and FDA should be held prior to the advisory committee meeting.
23 Nov 94	Letter to FDA confirming our 5 December 1994 meeting with the Division of Gastrointestinal and Coagulation; also included a proposed agenda and the list of BW attendees.
6 Dec 94	Letter from FDA requesting copies of Case Report Forms for the patients who were transplanted or withdrew from Study 49 and Study 50.
8 Dec 94	Telephone call from FDA to relay information that should be included in BW's package to the Advisory Committee, and what our presentations should be.
8 Dec 94	Telephone call from FDA to state that the diskette (dated October 1994) containing survival data for Studies 35/36, 37, 46 and 47, and the NIH registry is not readable; requested a new diskette in SAS PC format with additional information for Study 35/36, and detailed instructions on how to read the diskette.
12 Dec 94	As agreed in meeting with FDA on 5 December 1994, submitted responses to questions/comments resulting from the Medical Review of our NDA.

13 Dec 94	Telephone conversations with FDA Statistician concerning his request for a new diskett, in SAS PC format, containing survival data.
14 Dec 94	Letter from FDA with comments and requests concerning the NDA statistical review.
23 Dec 94	As requested by FDA in 6 December 1994 letter, submitted case report forms for patients who were transplanted or withdrew from Study 49 and Study 50.
23 Dec 94	Received by panafax, a copy of a letter The Upjohn Company received from the FDA advising that the Detroit District has recommended that the Center for Drug Evaluation and Research to approve the NDA.
5 Jan 95	Panafax received from FDA with additional statistical request for Studies 49 and 50.
11 Jan 95 and 12 Jan 95	Telephone call from FDA (11th) to request a meeting with BW on 30 January to demonstrate the randomization program used in Study 46 of the NDA clinical trials., follow–up telephone call from FDA (12th) to state that the Division Director approves of copies of the summary package being forwarded to the Advisory Committee prior to his comments.
11 Jan 95	Letter from FDA requesting attidional information for studies 49 and 50, to complete the NDA statistical review.
12 Jan 95	FDA Medical Reviewer comments of our 120–day Safety Update.
12 Jan 95	As discussed, submitted a draft of our Advisory Committee summary to the FDA for review and comment.
19 Jan 95	Received by panafax from FDA a letter requesting information pertinent to the NDA statistical review.
23 Jan 95	Telephone call from FDA to advise that review of our proposed Advisory Committee package has been completed.
24 Jan 95	Submitted response to FDA's 17 January 1995 request for information to complete the NDA statistical review.
25 Jan 95	Submitted a replacement diskette for diskette #1, entitled "Randomization program used in FLOLAN Study 46 with an empty data file, PAT46.DBF", originally submitted 24 January 1995 in response to their 17 January 1995 request.
25 Jan 95	As requested, submitted to FDA's District Office a copy of the original randomization program used in Study 46 and the version of program in which the study was written.
25 Jan 95	Telephone call to FDA to advise that requested diskettes for the randomization program has been forwarded, and BW Co. has received FDA comments concerning review of the Advisory Committee package.
27 Jan 95	Submitted response to FDA comments in letter of 17 October 1994, re: sterilization process validation provided in our original NDA. (A field copy was provided to the local FDA District Office.)

31 Jan 95	Telephone call to FDA to confirm their use of PPH data replacement diskettes submitted 25 January 1995.
1 Feb 95	Received by panafax from FDA Advisory Committee draft questions concerning NDA Studies 35/36 and 46.
1 Feb 95	Telephone call to FDA in response to FDA questions in 31 January 1995 telephone conversation, re: diskette version of randomization program.
2 Feb 95	Submitted by reference, update from Upjohn's Drug Master File 6065 data for Epoprostenol Sodium to our NDA.
3 Feb 95	Panafaxed to FDA, our comments to their draft questions received 1 February 1995.
3 Feb 95	Telephone call from FDA to request a diskette copy of our package insert in Word Perfect format.
6 Feb 95	As requested by FDA in 3 February 1995 telephone call, provided a diskette copy in Word Perfect format of the package insert identical to the insert submitted with the New Drug Application.
6 Feb 95	Provided a summary package to FDA to use in preparing for the 23 February 1995 Cardio-Renal Advisory Committee meeting.
9 Feb 95	Copies of final medical and statistical reviews picked up at FDA.
13 Feb 95	Telephone call to FDA to request meetings to resolve their concerns regarding validation of the randomization program used in Study 46, and to correct factual errors identified in the NDA review.
14 Feb 95	Telephone call from FDA to advise that a meeting is scheduled for 16 February 1995 to discuss issues related to the Advisory Committee.
14 Feb 95	Follow-up telephone call from FDA to request the purpose for the 16 February 1995 meeting between FDA/BW Co. scheduled prior to the Advisory Meeting.
14 Feb 95	Telephone call to FDA to request a meeting with the statisticians to discuss the adaptive randomization program.
15 Feb 95	Telephone call from FDA to postpone the 16 February 1995 meeting until 21 February 1995.
15 Feb 95	Telephone call from FDA to advise that a Statistical Meeting is scheduled for 21 February 1995, following the scheduled FDA/BW Co. meeting.
15 Feb 95	Return telephone call to FDA to discuss a meeting time with the statisticians to discuss the NDA.
23 Feb 95 and 24 Feb 95	Copy of questions presented to the Cardiovascular and Renal Drugs Advisory Committee members and their responses discussed during their 23 and 24 February 1995 meeting; also included are materials presented to the Advisory Committee members to support approval for the NDA.

6 Mar 95	Telephone call from FDA to request a background history of Patient 01012 entered into Study 46, and to confirm that BW is preparing a revised package insert, based on Advisory Committee comments; also discussed the status of the Chemistry Manufacturing and Controls, Microbiology, Pharmacology and Biopharmaceutics review.
6 Mar 95	Letter from FDA stating that the NDA chemistry, manufacturing and controls review is completed; requested explanations for questions concerning the drug substance and product.
13 Mar 95	Telephone conversation with FDA concerning the status of a PAI inspection at the Greenvile facility for the manufacture of the Sterile Diluent.
16 Mar 95	Telephone call from FDA concerning the status of Patient 01012 background history and the revised package insert, requested in their 6 March 1995 telephone call.
17 Mar 95	Telephone call to FDA to advise that the background history of Patient 01012 (Study 46), and the revised labeling is in preparation.
20 Mar 95	Submitted revised draft package insert with revisions based upon the Advisory Committee recommendations.
21 Mar 95	As requested by FDA in 6 March 1995 conversation, submitted the clinical case history of Patient 01012 entered in Study 46.
31 Mar 95	Telephone call from FDA to advise that a second deficiency letter with additional comments/questions has been forwarded to Upjohn on their Drug Master File.
10 Apr 95	Submitted response to the Chemistry, Manufacturing, and Controls comments in the 6 March 1995 letter from FDA.
12 Apr 95	As discussed with FDA in 12 April 1995 telephone conversation, submitted final printed container labeling for FLOLAN and the accompanying Sterile Diluent.
12 Apr 95	Telephone call from FDA to request data to support the acute and chronic dosing proposed in our labeling for children.
13 Apr 95	As requested in 12 April 1995 telephone conversation with FDA, submitted the demographic information (from clinical studies contained in our NDA) to support pediatric use of FLOLAN.
14 Apr 95	Minutes of a meeting with FDA to discuss specific items of the NDA.
20 Apr 95	As discussed in a 17 April 1995 telephone conversation with FDA, submitted a summary of mean change from baseline to maximum tolerated dose for each hemodynamic parameter in patients less that 16 years old (n=60) and greater than or equal to 16 years old (n=314) during acute dose ranging with FLOLAN.
20 Apr 95	As agreed in telephone conversation with FDA on 17 April 1995, submitted the corrected tables for a programming error in Study 46.

22 Apr 95	Received by panafax from FDA a draft letter stating that the product microbiological quality and sterility assurance information is complete and the sterilization process validation; requested a post-approval commitment.
24 Apr 95	Telephone call from FDA to request a post NDA commitment regarding the Microbiology section.
27 Apr 95	Telephone conference call with FDA in which the FDA requested a commitment from BW Co. to provide details of how the filter validation studies were conducted.
27 Apr 95	Provided by panafax to FDA a note stating that the patent information previously provided for our NDA should have included the 25 and 50 mg tablet strength, in addition to the 100, 150, 200 and 250 mg.
28 Apr 95	Letter from FDA listing their requests for filter validation information as discussed in the 27 April 1995 telephone conversation.
28 Apr 95	Letter from FDA with recommendations and requests pertaining to the NDA chemistry review.
28 Apr 95	As discussed with FDA in 12 April 1995 telephone conversation, submitted additional information in support of the pediatric indication for our NDA.
1 May 95	Telephone call from FDA to inform us that the NDA package had been sent to Dr. Temple's Office for his review and approval.
3 May 95	As discussed with FDA in 24 and 27 April 1995 telephone conversations, letter to FDA with a post-approval commitment to provide additional information concerning sterilization process validation information.
4 May 95	Letter from FDA requesting current NDA safety information.
5 May 95	Telephone call from FDA to request a safety update to our NDA.
9 May 95	Letter from FDA stating that our NDA is approvable; requested final printed labeling and our response to their 28 April 1995 request for additional chemistry information.
12 May 95	Letter to FDA stating that a response to their comments in the 9 May 1995 approvable letter will be submitted in the near future.
22 May 95	Submitted a revised draft package insert in response to the 9 May 1995 NDA approvable letter.
31 May 95	Telephone call from FDA to request the number of pediatric patients in the acute dose studies who had Primary Pulmonary Hypertension, and data to support the chronic benefit.
2 Jun 95	As requested, provided FDA with the number of patients who had primary pulmonary hypertension.
6 Jun 95	In response to the 9 May 1995 approvable letter, submitted final printed container labeling for FLOLAN and the accompanying Sterile Diluent.

7 Jun 95	In response to FDA telephone requested of 30 May 1995, submitted a copy of Protocol 46 and Protocol 47.
9 Jun 95	Submitted additional comments concerning placement of lot numbers and expiration dates on final printed container labeling.
9 Jun 95	Letter to FDA providing our response to their comments contained in 28 April 1995 letter.
22 Jun 95	Submitted the approval safety update.
7 Jul 95	Submitted the user fee payment to cover the balance of the application fee for review.
11 Jul 95	Telephone call from FDA requesting that we submit a new Methods Validation package to incorporate the revisions which have occurred as a result of the CMC review of the pending NDA.

24 Jul 95 Letter to FDA incorporating amended DMF from Upjohn to NDA

27 Jul 95 Internal communication Dr Radeschi's request to fax pt's CREST

9 aug 95 Letter from FDA acknowledging receipt of 7/24/95 letter

23 aug 95 Letter to FDA conforming data in Table CHD [illegible]

[illegible] to FDA [illegible]

28 [illegible] Letter from FDA [illegible]

7 Jun 95	In response to FDA telephone requested of 30 May 1995, submitted a copy of Protocol 46 and Protocol 47.
9 Jun 95	Submitted additional comments concerning placement of lot numbers and expiration dates on final printed container labeling.
9 Jun 95	Letter to FDA providing our response to their comments contained in 28 April 1995 letter.
22 Jun 95	Submitted the approval safety update.
7 Jul 95	Submitted the user fee payment to cover the balance of the application fee for review.
11 Jul 95	Telephone call from FDA requesting that we submit a new Methods Validation package to incorporate the revisions which have occurred as a result of the CMC review of the pending NDA.

24 Jul 95 Letter to FDA incorporating amended DMF from UPJohn to NDA

27 Jul 95 Internal communication Dr Radeschi's request to tx pt ε CREST

9 aug 95 Letter from FDA acknowledging receipt of 7/24/95 letter

23 aug 95 Letter to FDA confirming data in Table (HD ✓ chronic Admin) are correct

9 Sep 95 - tel call from CSO that NDA signed by Dr Fried + deliv to Temple

all to FDA 15 Sep 95 - Temple had not completed review

20 Sep 95 approval

28 Sep 95 Letter to FDA amending Methods Validation pkg

EXHIBIT 8

PATENT TERM EXTENSION CALCULATIONS
for U.S. PATENT 4,883,812

EXHIBIT 8 U.S. 4,883,812

Patent Term Extension Calculations

IND Effective Date: **6/29/79**

6/29/79 - 11/27/89

Patent Issue Date: **11/28/89**

11/28/89 - 12/31/89 = 33
1/1/90 - 12/31/90 = 365
1/1/91 - 12/31/91 = 365
1/1/92 - 12/31/92 = 366
1/1/93 - 12/31/93 = 365
1/1/94 - 2/27/94 = 58
1552 x 0.5 = 776 days

NDA Submission Date: **2/28/94**

2/28/94 - 12/31/94 = 307
1/1/95 - 9/20/95 = 263
570 x 1 = 570 days

NDA Approval Date: **9/20/95**

776 + 570 = 1346 days

Patent Term Extension + Expiration as terminally disclaimed against U.S. 4,539,333

Expiration as terminally disclaimed against U.S. 4,539,333: **9/3/02**

9/3/02 - 12/31/02 = 119
1/1/03 - 12/31/03 = 365
1/1/04 - 12/31/04 = 366
1/1/05 - 12/31/05 = 365
1/1/06 - 5/11/06 = 131
1346 days

Expiration as terminally disclaimed against U.S. 4,539,333 + 1346 day Patent Term Extension: **5/11/06**

14 yr Cap from NDA Approval Date

NDA Approval Date: **9/20/95**

9/20/95 + 14 yrs = 9/20/09

14 year Patent Term Cap Date: **9/20/09**

EXHIBIT 9

U.S. PATENT 4,335,139

United States Patent [19]

Watts et al.

[11] **4,335,139**

[45] **Jun. 15, 1982**

[54] **PHARMACEUTICAL FORMULATIONS CONTAINING PROSTACYCLIN COMPOUNDS**

[75] Inventors: **Ian S. Watts, Sidcup; Peter H. Marsden, Dartford, both of England**

[73] Assignee: **Burroughs Wellcome Co., Research Triangle Park, N.C.**

[21] Appl. No.: **182,054**

[22] Filed: **Aug. 28, 1980**

Related U.S. Application Data

[63] Continuation of Ser. No. 39,645, May 16, 1979, abandoned.

[30] **Foreign Application Priority Data**

May 17, 1978 [GB] United Kingdom 20175/78

[51] **Int. Cl.**[3] **A61K 31/34**

[52] **U.S. Cl.** **424/285**

[58] **Field of Search** 424/305, 317, 285

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,058,623 11/1977 Rolf-Rudiger

FOREIGN PATENT DOCUMENTS

2654149	6/1977	Fed. Rep. of Germany .
2720999	11/1977	Fed. Rep. of Germany .
2351112	4/1977	France .
1489780	10/1977	United Kingdom .
1503447	3/1978	United Kingdom .
1504070	3/1978	United Kingdom .
1504437	3/1978	United Kingdom .

OTHER PUBLICATIONS

Hayashi et al.—Chem. Abst., vol. 90 (1979), pp. 127, 526.

Shirley—Organic Chemistry (1964, Holt), pp. 535-536.

Finar—Organic Chemistry—3rd Edit. (Longmans, 1959), pp. 305-307.

Primary Examiner—Sam Rosen

Attorney, Agent, or Firm—Donald Brown

[57] **ABSTRACT**

Stabilized pharmaceutical formulations of prostacyclin or certain analogues thereof comprising an amino acid buffer, optionally containing a base, and the preparation of such formulations.

34 Claims, No Drawings

PHARMACEUTICAL FORMULATIONS CONTAINING PROSTACYCLIN COMPOUNDS

This is a continuation of application Ser. No. 039,645, filed May 16, 1979, now abandoned.

The present invention relates to pharmaceutical compositions containing prostacyclin (PGI_2, PGX), 15-methylprostacyclin, 16,16-dimethylprostacyclin, or their pharmaceutically acceptable salts.

Prostacyclin and its salts are important in human medicine and veterinary practice as they have a powerful anti-aggregating action on blood platelets and also accelerate wound healing and prevent, or have a therapeutic effect on, stomach ulcers.

The anti-aggregatory effect on blood platelets is useful, for example, in preventing or mitigating the formation of thrombi or emboli during extracorporcal circulation of blood, e.g. in renal dialysis and cardio-pulmonary by-pass.

However, a difficulty experienced with prostacyclin and its salts is that prostacyclin and its salts are unstable, especially in aqueous solution, and are quickly transformed into 6-oxo-$PGF_{1\alpha}$ and its salts, which are almost pharmacologically inactive and have very few, if any, of the beneficial activities of prostacyclin and its salts.

Prostacyclin and its salts are known to be more stable in alkaline media than in acidic or neutral media. Although some improvement in stability has been obtained by using tris buffer in alkaline solution, the prostacyclin has still been rapidly decomposed. This has meant that the prostacyclin has had to be made up very shortly before use by, say, intravenous infusion, and the pharmacological activity of the solution has changed considerably while it has been waiting to be used. This has made it difficult for the infusion to be controlled as carefully as desirable.

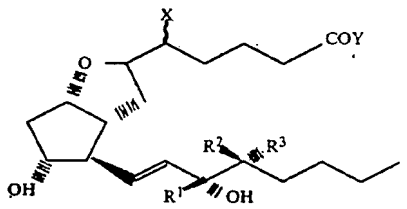
We have now surprisingly found that a marked improvement in stability can be obtained by having the prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin, or a salt thereof, preferably the sodium salt, (hereinafter referred to as the "active compound"), in association with a pharmaceutically acceptable buffer having a pH value of at least 9 and based on an amino acid as the principal buffering acid in the buffer. The active compound and the buffer may be in association in the solid state and in solution in a solvent, usually water. When the active compound and buffer are in solution, the pH measured is that of the solution containing said active compound and buffer, and said formulation or buffer preferably has a pH of at least 9.

If the active compound and the buffer are in association in the solid state, by pH value of the buffer or the formulation we mean the pH measured in any one of the following ways. If the solid is a frozen solution, then the pH measured is that of the solution resulting from thawing the frozen solution. For other solid states, the pH measured is that of the solution resulting from dissolving the associated active compound and buffer in Water for Injections having a pH value of 7. For the particular case of a freeze dried residue of such a solution, the pH measured is that of the solution resulting from dissolving a sample of the residue in the minimum volume of Water for Injections having a pH value of 7 required to produce a clear solution.

In the solid state, the freeze drying of such a solution results in improved stability of the active compound. Freeze drying may be effected in the conventional manner in, for example, an ampoule or vial. The solution may also be frozen and stored at, say, −20° C. for use as a frozen injection or for diluting on thawing.

Such a solution and all solutions hereinafter referred to are, for medicinal purposes, to be understood to be sterile solutions.

Prostacyclin and analogues thereof, in particular 15-methylprostacyclin and 16,16-dimethylprostacyclin, and a salt of one of these may be prepared by methods described in our co-pending UK Patent Application No. 19384/76 for the preparation of compounds of analogous structure. These include dehydrohalogenation of a compound of formula (I):



(I)

wherein X is bromo or iodo; Y is OH, NHR^4 or OR, R being alkyl of 1 to 4 carbon atoms or a pharmaceutically acceptable cation such as sodium, R^4 being alkyl of 1 to 4 carbon atoms; R^1, R^2 and R^3 are independently selected from hydrogen and alkyl of 1 to 4 carbon atoms particularly methyl, with a base; for example, when R^1 is hydrogen R^2 and R^3 are both methyl and vice-versa; and converting, if necessary, the resulting compound in which Y is NHR^4 or OR, R and R^4 being alkyl of 1 to 4 carbon atoms, into the desired active compound.

The amino-acid for use in the buffer is preferably sulphur-free and the most preferred amino-acid is glycine, especially as it is readily available and, if prepared synthetically, does not need to be optically resolved; but other amino-acids such as valine, alanine and arginine may also be used.

The total concentration of amino-acid (i.e. including its salts) in the buffer is preferably as low as is consistent with obtaining a stable buffer, e.g. in the range of from 0.02 M to 0.03 M, preferably about 0.025 M, as the presence of too much amino-acid tends to reduce the stability of the active compound by increasing the ionic strength of a solution of the associated active compound and buffer. The amino-acid must be sufficiently soluble to provide the necessary buffering capacity.

If sodium chloride is present in a solution of the buffer, as is preferred, the amount added should not be such that the solution is pharmaceutically unacceptable. The molar concentration of sodium chloride is preferably about the same as that of the amino-acid. Too much sodium chloride would raise the ionic strength of a solution of the associated active compound and buffer undesirably and adversely affect the stability of the active compound. Other salts, e.g. potassium chloride, may also be present if they, or their amounts, are pharmaceutically acceptable.

The pH of the buffer is preferably 10.2 to 11.6, especially about 10.5, when measured by the appropriate method as hereinabove described.

As an example of preparing a buffer solution according to the invention containing prostacyclin, a solution of glycine in water and also containing some sodium chloride was prepared. To this solution was added so-

dium hydroxide as base to raise the pH to the desired level and then the prostacyclin was added.

Although sodium hydroxide was used as base in the above example, any base may be used that is strong enough to give a buffer solution of the desired pH. Naturally the base should be one that gives rise to a pharmaceutically acceptable solution, i.e. one that is not deleterious to the recipient. The amount of amino-acid, for example, glycine and base, for example, sodium hydroxide, used should be as little as is necessary to stabilize the active compound for the period of time required. Use of excess of either or both amino-acid or base results in retention of water in a freeze dried product which brings about deterioration of the active compound. However, the pH of the solution is an important factor in assessing pharmaceutical acceptability. If the buffer solution is to be introduced into a machine for example in renal dialysis, then the pH can be up to 12 or even more, but if the buffer solution is to be administered in a large volume into a vein, for example in cardio-pulmonary by-pass, then the pH on entering the vein should preferably be in a range of from 8.4 to 9, and for this the pH of the buffer solution can be lowered shortly before use.

Other buffering agents may be present in the buffer solution but their amount should not substantially reduce the stability of the active compound in the solution. For example, some carbonate may be present, derived from the prostacyclin, e.g. prostacyclin sodium may contain up to 5% by weight of sodium carbonate.

As an active compound is very active pharmacologically, the amount of it needed is very small; for example only a few milligrams are needed for a one hour infusion into an average person of 70 kg body weight. The amount of active compound present in a given buffer solution before freeze drying depends on the projected use for the freeze dried material on reconstitution. The reconstitution may be with buffer solution free of active compound so that the ratio of active compound to buffering constituents may be much greater than in the solution used for administration.

If a solution containing only buffering agents, active compound and sodium chloride is freeze dried, the physical strength and appearance of the freeze dried plug obtained are not particularly satisfactory. It is accordingly preferred to include an excipient in the buffer solution before freeze drying the solution. The preferred excipient is mannitol. Preferably the concentration of excipient is from 25 to 50 mg/ml of buffer solution. For mannitol if less than 25 mg/ml is used there is insufficient improvement in strength and appearance. If more than 50 mg/ml is used there is little or no further improvement and the stability of the active compound may be adversely affected. The excipient provides, in the freeze dried plug, a supporting matrix and improves the physical strength and appearance of the plug. Not all excipients may be used; for example those producing excessive foaming of the reconstituted material in the freeze drying vial, e.g. polyvinylpyrrolidone, should be avoided. Also, others affecting the pH of the buffer, e.g. glycine itself, should be avoided.

The buffer solution may also contain, if desirable, other therapeutic material besides the active compound. A freeze dried product may also contain such other therapeutic material or these may be added to the reconstituted buffer solution. Such other therapeutic material may partly or completely replace the excipient.

The solvent used in preparing the buffer solution is preferably Water for Injections (European Pharmacopeia) or other water suitable for use in infusions or injections. When reconstituting the freeze dried material for use, it may be redissolved in Water for Injections generally having a pH value in the range of from 5.5 to 7, preferably 7, or in amino-acid buffer solution having a pH of at least 9, preferably about 10.5, or perhaps some of the buffer solution may be added to a solution in Water for Injections. Reconstitution of the freeze dried material may also take place by dissolving it in an infusion base, such as physiological saline suitable for infusion. It is also possible to use glucose for this purpose.

The freeze drying of the buffer solution may be carried out in any conventional manner, with the water content of the plug being lowered as far as convenient to improve further the stability of the active compound.

The amount of active compound required for therapeutic effect varies with the route of administration. In general, a suitable dose for a mammal will lie in the range of from 0.01 to 200 mg. per kilogram body weight, conveniently of from 0.01 to 10 mg/kg, preferably of from 0.1 to 1.0 mg/kg, and especially of from 0.2 to 0.5 mg/kg.

The amount of active compound present in an ampoule for administration by infusion will lie in the range of from 0.1 to 1.5 mg/kg, preferably of from 0.5–1.0 mg/kg. When used in man, the active compound may be several times more potent than in other mammals, and accordingly it may be desirable to use doses which appear at the lower ends of the dose ranges given hereinabove.

For human and veterinary use, it may be convenient to provide a collection of at least two vessels, for example as a multicomponent pack; one of which is a vial or ampoule containing a freeze dried (lyophilised) plug of the buffered active compound as described hereinabove, another vessel of which is a vial or ampoule containing a further amount of the buffer in aqueous solution or freeze dried which does not contain the active compound. The freeze dried product may then be reconstituted with the aqueous buffer, or where the contents of the second vessel are freeze dried, with a suitable aqueous diluent from a third vessel. The reconstituted material may then be diluted further, if required, to provide the desired dosage immediately prior to administration. Thus a freeze dried preparation of, for example 0.5 mg active compound, at pH 11.5 may be diluted, with 50 or 500 ml of aqueous dextrose or saline solution having a pH such that the resultant solution has a pH of 10.0 to 10.5.

Accordingly the present invention provides at least the following:

(a) A pharmaceutically acceptable buffer solution which comprises prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin or a salt of any one of these, and, as principal buffering acid, an amino-acid, the solution having a pH of at least 9;

(b) A method of preparing a buffer solution according to (a), which comprises bringing the ingredients into solution in a suitable solvent;

(c) A freeze dried material obtained by freeze drying a buffer solution according to (a);

(d) A frozen injection obtained by freezing a buffer solution according to (a);

(e) A solution suitable for injection or infusion obtained by dissolving a freeze dried material according to

(c) in a suitable solvent, or thawing a frozen injection according to (d);

(f) A method for administering prostacyclin, 15-methylprostacyclin 16,16-dimethylprostacyclin, or a salt of any one of these which comprises administering a solution according to (a), (d) or (c).

(g) A pharmaceutical formulation comprising an active compound selected from prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin and a salt of any one of these in association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on an amino acid as principal buffering acid in the buffer.

(h) A pharmaceutical formulation comprising an active compound selected from prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin and a salt of any one of these in association with a pharmaceutically acceptable buffer based on an amino acid as the principal buffering agent.

The present invention is illustrated by the following Examples:

EXAMPLE 1

Sterile solutions containing prostacyclin (0.2 and 0.4 mg/ml) and mannitol (50 mg/ml) were prepared in the following buffers: glycine, orginine, valine, alanine, carbonate, and tris. The concentrations of the buffering components were:

Amino acid buffer: 0.025 M amino acid, 0.025 M sodium chloride and sodium hydroxide q.s. pH 10.5.

Carbonate buffer: 448 mg/l sodium bicarbonate and 1614 mg/l sodium carbonate.

Tris buffer: 6054 mg/l of tris base and 2.5 ml of 0.1 N sodium hydroxide per liter.

5 ml portions of each of these solutions were then freeze dried in vials by freezing at −40° C. and under vacuum, carrying out first stage drying at 0° C. and second stage drying at 20° C. Sealing of the vials was conducted in a nitrogen atmosphere. The freeze dried products were then subjected to an accelerated storage test at the temperature shown in Table I. The products obtained after the times indicated in Table I were analysed for their prostacyclin content by high performance liquid chromatography (HPLC). The HPLC was carried out on freeze dried preparations reconstituted in 10 ml of 0.025% tetramethylammonium hydroxide solution.

The column used was a 25 cm×4.2 mm I.D. stainless steel one filled with laboratory prepared Partisil ODS packing material made as given below. The column was packed using the method of Webber and McKerrel, J. Chromatog., 122, 243, (1976), employing carbon tetrachloride as the slurry medium.

The column packing material was prepared as follows; 10 μm Partisil (10 g) was dried at 80° C. in vacuo for two and half hours in a 250 ml round bottomed flask. Octadecyltrichlorosilane (10 ml) and dry toluene (100 ml) were added and the solution was refluxed for three hours with paddle stirring using a reflux condenser fitted with a calcium chloride guard tube. The mixture was allowed to cool and then filtered through a 0.5 μm millipore filter. The silica in the filter was washed with 250 ml methanol, slurrying the solid continuously, then with 250 ml hot acetone and dried at 80° C. in vacuo for about two hours. The product (11 g) was treated with trimethylchlorosilane (10 ml) as above, refluxing for 45 minutes to give the final product.

The mobile phase used was water (1200 ml) in which was dissolved 5 g boric acid and 7.6 g di-sodium tetraborate and then methanol (800 ml) was added. The column temperature used was ambient i.e about 25° to 30° C., and the mobile phase flow rate was 3.6 ml/min. and the pressure used was 20 MPa. Detection of the products was carried out using a Pye Unichem LC3 at 205 nanometers wavelength, 0.16 aufs (absorbance units full scale) for 0 to 100 μg/ml solutions.

The amount of prostacyclin present was determined by peak height measurement and comparison with a reference sample of known concentration.

The results obtained are set out in Table I.

TABLE I

	HPLC assay (% prostacyclin remaining)				
	66 hours				6 days (144 hours)
Buffer	70° C.	60° C.	50° C.	37° C.	26° C.
Glycine Arginine Valine Alanine	0	0	3	90	96
Carbonate	0	2	3.5	45	62
Tris	0	0	trace	trace	35

It can be clearly seen from Table I that at normal storage temperatures, i.e. about 37° C. or below, the amino-acid buffers, especially glycine, are superior to the other two buffers tested. Although the carbonate buffer is inferior to the amino-acid buffer it is clear that some carbonate could be tolerated in the amino-acid buffer.

EXAMPLE 2

Freeze dried Injection of prostacyclin (1mg)	
Prostacyclin	1.000 mg
Mannitol	50.000 mg
NaCl (0.025M)	2.932 mg
Glycine (0.025M)	3.760 mg
NaOH	q.s. to pH 10.5

Using the general procedure give in Example 1, a 1 mg prostacyclin injection comprising the above ingredients was freeze dried to give a residue which may contain up to 5% w/w of water.

EXAMPLE 3

A stirred solution of $PGF_{2\alpha}$ methyl ester (50 mg) in ether (1 ml) was treated with sodium bicarbonate (115.0 mg; 10 molecular equivalents) and water (1 ml) and then dropwise during 2 hours with aqueous potassium triiodide (0.7 molar; 0.261 ml). After stirring overnight, the reaction mixture was shaken with ether and aqueous sodium thiosulphate; the etheral phase was separated, washed with water, dried with magnesium sulphate, and evaporated to leave a yellow gum of 5ξ-iodo-9-deoxy-6ξ,9α-epoxyprostaglandin $F_{1\alpha}$ methyl ester.

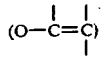
A solution of 5ξ-iodo-9-deoxy-6ξ,9α-epoxyprostaglandin $F_{1\alpha}$ methyl ester (100 mg) in methanolic sodium methoxide prepared from sodium (46 mg) and dry methanol (0.70 ml) was set aside under dry nitrogen for 5 hours, then freed from solvent in high vacuum. The residual amorphous solid was washed with benzene, set aside in the air overnight, and stirred with N aqueous sodium hydroxide (0.5 ml) to give a suspension of colourless fine needles. The crystals were collected, washed with a few drops of N aqueous sodium hydroxide, and dried in the air to give the sodium salt of 9-deoxy-6,9α-epoxy-Δ^5-prostaglandin $F_{1\alpha}$. The inhibition

of arachidonic acid-induced aggregation of human platelets at a concentration of 0.2 ng/ml by this salt and its instability in water at acid pH, together with further evidence, is compatible with assignation of the configuration (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin $F_{1\alpha}$.

The high-resolution ^{13}C n.m.r. spectrum of a solution of the crystals in dimethyl sulphoxide-d_6 showed the expected 20 resonances whose chemical shifts were entirely consistent with the chemical structure established for Prostacyclin. No impurity peaks were detected.

EXAMPLE 4

5ξ-Iodo-9-deoxy-6ξ,9ξ-epoxyprostaglandin $F_{1\alpha}$-methyl ester (500 ml) was stirred with methanolic NaOMe prepared from Na (0.23 g, 10 equivs.) and MeOH (3.5 ml) under N_2 at room temperature overnight; 1 N aq. NaOH (2.5 ml) was added to the yellow reaction solution to bring about hydrolysis of the ester moiety and, after 2 hours, the methanol was evaporated in vacuo at room temperature. The residual aqueous solution gave rise spontaneously to a mass of colourless fine needles of the desired sodium salt which was cooled (0°), collected, washed sparingly with 1 N aq. NaOH, air-dried, and stored in a stoppered tube; this salt (383 mg) had ν max (KBr disc) 1692 cm^{-1}

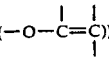


and twenty ^{13}C resonances only were observed at 182.7 (C-1), 158.2 (C-6), 140.0 and 134.3 (C-13,14), 100.7(C-5), 87.5(C-15), 80.6 and 75.5(C-9,11), 58.0(C-12), 49.0, 45.8, 42.4, 41.9, 37.5, 35.8(C-18), 31.6, 29.9, 29.3, 26.7(C-19), 18.4(C-20) ppm from TMS in DMSO-d_6). The product was sodium (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin $F_{1\alpha}$ (syn. sodium prostacyclin).

EXAMPLE 5

5ξ-Iodo-9-deoxy-6ξ,9α-epoxyprostaglandin $F_{1\alpha}$ methyl ester was treated with 1,5-diazabicyclo-5-nonene (DBN) at room temperature in the absence of a solvent for a few hours.

The DBN and hydrogen iodide were conveniently removed by adsorption on to a column of SiO_2, prepared from a suspension of SiO_2 in EtOAc/Et_3N 50:1, and the vinyl ether was eluted with the same solvent system. I.R. spectroscopy (thin film, ν max 1738 (CO_2Me) and 1696 cm^{-1}



^1H n.m.r. in C_6D_6—Et_3N, 19:1 (δ4.22, triplet of triplets[12], J6.9 and 1.0 Hz (C-5 vinyl proton)), and ^{13}C n.m.r. in C_6D_6—Et_3N, 19:1 (distinctive features were resonances at 159.8 (C-1), 155.8 (C-1), 155.8(C-6), 137.2 and 130.6(C-13,14), 95.3(C-5), 84.1(C-15) 77.3 and 72.2 (C-9,11) and 51.1 (Me ester) ppm from TMS).

The vinyl ether (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin methyl ester, was hydrolysed with aqueous sodium hydroxide to give synthetic sodium prostacyclin.

EXAMPLE 6

(5R, 6R)-5-Iodo-PGI_1 methyl ester (13.375 g, containing ca.2% 5S, 6S isomer) was taken up in methanolic sodium methoxide [from Na (6.23 g) and MeOH (94 ml)] at room temperature under N_2 and set aside at room temperature overnight. The resulting yellow solution was treated with 1 N aqueous NaOH (70 ml), filtered from sediment, set aside at room temperature for 2 hours, and freed from MeOH on a Buchi evaporator in vacuo at room temperature. The residual syrup was treated with H_2O (25 ml) and with more 1 N aqueous NaOH (80 ml), crystallisation taking place spontaneously to give a mass of felted crystals. After cooling to 0°, the solid was collected, washed with ice cold 1 N aqueous NaOH (ca. 40 ml) until the washings were colourless, and dried in the air (2 days) to constant weight, affording 10.15 g, m.p. 164°-166° (following drying at 100°) of colourless prostacyclin sodium salt.

EXAMPLE 7

Sterile Diluent for Injection of Prostacyclin		
Glycine (0.025M)		94.0mg
NaCl (0.025M)		73.3mg
NaOH	q.s. to pH	10.5
Water for Injections up to		50ml.

Using the general procedure described in Example 1, the above ingredients were used in a solution for use as a diluent.

We claim:

1. A pharmaceutical formulation comprising an active compound selected from prostacyclin, 15-methylprostacyclin, 16, 16-dimethylprostacyclin or a pharmaceutically acceptable salt of any one of these in association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on a pharmaceutically acceptable amino acid as a buffering acid in the buffer and, optionally, a further pharmaceutically acceptable carrier.

2. A formulation according to claim 1, wherein the further pharmaceutically acceptable carrier is or includes a solvent which dissolves the active compound and the buffer, to form a solution.

3. A formulation according to claim 1, wherein the further pharmaceutically acceptable carrier is or includes water.

4. A formulation according to claim 1, wherein a solution containing the active compound and the buffer is suitable for injection or infusion.

5. A formulation according to claim 1, wherein a solution containing the active compound and the buffer is freeze dried or frozen.

6. A formulation according to claim 1, wherein the amino-acid is sulphur-free.

7. A formulation according to claim 1, wherein the amino-acid is selected from the group consisting of glycine, alanine, arginine and valine.

8. A formulation according to claim 1, wherein the total concentration of the amino-acid including its salts, is in the range of from 0.02 to 0.03 M.

9. A formulation according to claim 1, wherein the total concentration of the amino-acid, including its salts, is about 0.025 M.

10. A formulation according to claim 1, wherein the further pharmecutically acceptable carrier comprises sodium chloride.

11. A formulation according to claim 1, wherein the pH of the buffer is in the range of from 10.2 to 10.8.

12. A formulation according to claim 1, wherein the further pharmaceutically acceptable carrier comprises an excipient.

13. A formulation according to claim 1, wherein the further pharmaceutically acceptable carrier comprises mannitol.

14. A formulation according to claim 1, wherein the active compound is prostacyclin or a salt thereof.

15. A formulation according to claim 1, wherein the salt is a sodium salt.

16. A formulation according to claim 1, wherein the active compound is prostacyclin sodium salt.

17. A formulation according to claim 1, comprising prostacyclin sodium salt in association with glycine and optionally, a further pharmaceutically acceptable carrier.

18. A method of preparing a pharmaceutical formulation according to claim 1, which comprises bringing an active compound selected from prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin, and a salt of any one of these into association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on an amino-acid as principal buffering acid in the buffer and, optionally, a further pharmaceutically acceptable carrier.

19. A formulation according to claim 1, when prepared by a method according to claim 18.

20. A method of inhibiting the aggregation of platelets, which comprises the bringing of said platelets into association with an amount of a formulation according to claim 1 containing an effective platelet aggregation inhibitory amount of the active compound.

21. A method of inducing vasodilation in a mammal, which comprises the administration to said mammal of an amount of a formulation according to claim 1 containing a non-toxic, effective vasodilatory amount of the active compound.

22. A method for the treatment or prophylaxis of thrombosis in a mammal or mammalian tissue which comprises administration of an amount of a formulation according to claim 1 containing a non-toxic, effective antithrombotic amount of the active compound.

23. A method of lowering blood pressure in a mammal which comprises administration to said mammal of an amount of a formulation according to claim 1 containing a non-toxic, effective hypotensive amount of the active compound.

24. A method for the treatment or prophylaxis of a gastric lesion in a mammal which comprises administration to said mammal of an amount of a formulation according to claim 1 containing a non-toxic, effective therapeutic or prophylactic amount of the active compound.

25. A method of stabilizing an active compound selected from the group consisting of prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin and a pharmaceutically acceptable salt of any one of these against hydrolysis of the enol ether moiety, which method comprises bringing said compound selected from the group consisting of prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin, and a pharmaceutically acceptable salt thereof into association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on an amino acid as a buffering acid in the buffer.

26. A pharmaceutical formulation comprising an active compound selected from prostacyclin, 15-methylprostacyclin, 16,16-dimethylprostacyclin and a salt of any one of these in association with a pharmaceutically acceptable buffer based on an amino acid as principal buffering agent and buffered to a pH of at least 9.

27. A formulation according to claim 26, wherein the formulation further includes a base.

28. A pharmaceutical composition comprising prostacyclin or a pharmaceutically acceptable salt thereof in association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on an amino acid as the principal buffering acid in the buffer.

29. The composition of claim 28 in which the amino acid is arginine.

30. The composition of claim 28 in which the amino acid is valine.

31. The composition of claim 28 in which the amino acid is alanine.

32. The composition of claim 28 in a form for injection, said composition including water.

33. A pharmaceutical composition comprising the sodium salt of prostacyclin in association with a pharmaceutically acceptable buffer having a pH of at least 9 and based on glycine as the acid in the buffer.

34. The composition of claim 33 in a form for injection, said composition including water.

* * * * *

EXHIBIT 10

U.S. PATENT 4,539,333

United States Patent [19]

Moncada

[11] Patent Number: 4,539,333

[45] Date of Patent: Sep. 3, 1985

[54] **PROSTACYCLIN, METHODS OF USING AND METHOD OF MAKING**

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[30] Foreign Application Priority Data

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[52] U.S. Cl. 514/469; 435/63; 549/465

[58] Field of Search 260/346.22; 424/285; 435/63; 549/465

[56] References Cited

U.S. PATENT DOCUMENTS

4,158,667 6/1979 Axen 562/503
4,338,325 7/1982 Johnson et al. 549/465

OTHER PUBLICATIONS

Pace-Asciak et al., Biochemistry, vol. 10, No. 20, (1971), pp. 3657-3664.

Corey et al., J.A.C.S., 99(6), Mar. 16, 1977, pp. 2006-2008.

Johnson et al., Prostaglandins, vol. 12(6), Dec. 1976, pp. 915-928.

Pace-Asciak et al (II), Prostaglandins, Sep. 1978, vol. 16, No. 3, pp. 397-410.

Shirley, Organic Chemistry, Holt, Rinehart and Winston, (1946), p. 353.

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Attorney, Agent, or Firm—Donald Brown

[57] **ABSTRACT**

Prostacyclin, its salts, biosynthesis and synthesis thereof, pharmaceutical formulations containing them, and their use in medicine.

34 Claims, No Drawings

PROSTACYCLIN, METHODS OF USING AND METHOD OF MAKING

This invention relates to the extraction, isolation and synthesis of a prostaglandin derivative, its use as a chemical intermediate, formulations containing it, and its use in medicine.

Prostaglandin endoperoxides (PGG_2 and PGH_2) are generated from arachidonic acid by a membrane-bound cyclo-oxygenase enzyme system, described by Hamberg and Samuelsson (Biochem. Biophys. Acta. 326, 448–461, 1974) and are subsequently transformed to $PGF_{2\alpha}$, PGE_2, PGD_2, or Thromboxane A_2. Thromboxane A_2 shares with the prostaglandin endoperoxides the important biological properties of contracting strips of rabbit aorta and aggregating blood platelets.

The Applicants have now found that microsomes derived from a variety of mammalian tissues catalyse the enzymic transformation of the prostaglandin endoperoxides to a prostaglandin derivative (hereinafter referred to as Prostacyclin which does not contract strips of rabbit aorta, relaxes strips of rabbit coeliac mesenteric and coronary arteries, has a potent anti-aggregatory action on blood platelets, is a strong vasodilator in whole animals and has other properties described hereinafter.

Microsomes derived from rabbit or pig blood vessels such as veins and arteries, and rat stomach fundus produce about 80–90% conversion of the prostaglandin endoperoxides. Microsomes derived from rabbit lung tissue and rat pyloric tissue produce 25% conversion of the prostaglandin endoperoxides, whereas those derived from rat kidney, brain, spleen, liver, heart and seminal vesicle tissues produce 5% or less conversion of the prostaglandin endoperoxides.

Prostacyclin is unstable at room temperature in aqueous medium, having a half-life of approximately 10 mins., but its anti-aggregatory activity can be preserved for several days by dissolving the substance in aqueous alkali or in dry acetone and storing at −20° C. On average prostacyclin is 10–40 times more potent as an anti-aggregatory agent than PGE_1 and 5–20 times more potent than PGD_2, itself a potent inhibitor of platelet aggregation. Prostacyclin also interrupts and reverses the presence of platelet aggregation.

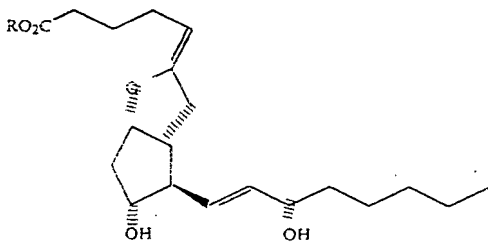
Prostacyclin may be prepared biosynthetically by incubating PGG_2 or PGH_2 with aortic microsomes in a suitable buffer solution such as Tris buffer, for approximately 2 minutes at a temperature in the region of 22° C. Conversion of the prostaglandin endoperoxides is approximately 85%.

Extraction of Prostacyclin is achieved by the addition of cold (0° C.) dry diethyl ether to the incubation mixture. The addition of cold ether stops the enzyme reaction and Prostacyclin enters the ether phase which may be separated from the aqueous phase. Evaporation of the ether by standard techniques such as bubbling nitrogen through the solution results in the Prostacyclin being left as a residue which may subsequently be resuspended in an aqueous solution for further examination or dissolved in anhydrous acetone and stored at a temperature in the region of −20° C. for future use.

The aortic microsomes employed in the incubation mixture may be extracted from pig or rabbit aortas. Aortas may be frozen solid preferably by dropping them in liquid nitrogen, and then crushed to form a powder which is resuspended in a suitable buffer solution and subsequently homogenized. The homogenate may then undergo sequential centrifugation so as to isolate the microsomal fraction which may be resuspended in deionized water and lyophilized. The incubation mixture was shown to have an immediate anti-aggregatory effect by monitoring the aggregation of human blood platelets in a Born aggregometer.

Prostacyclin may be prepared using other tissues identified above in substantially a similar manner. Prostacyclin formed in such an incubation mixture appears to be different from the other products of PG endoperoxides so far described. Its biological properties on the isolated tissues, its instability and its potent anti-aggregatory activity show that Prostacyclin is not being PGE_2 or $PGE_{2\alpha}$. The presence of prostaglandin D_2 isomerase in homogenates of several tissues has been described. As prostacyclin is unstable and is a more potent anti-aggregatory agent than PGD_2, it cannot be regarded as PGD_2. Furthermore, PGD_2, isomerase is present in the 100,000 g. supernatant, a fraction that did not produce Prostacyclin from PG endoperoxides. Moreover, PGD_2 isomerase needs glutathione as a co-factor and the incubations were carried out in the absence of cofactors. PGE_2, $PGF_{2\alpha}$ and PGD_2 were not substrates for aortic microsomes and therefore 15-keto PGs and other products of prostaglandin catabolism could not be considered as Prostacyclin. Prostacyclin is also unlikely to be a known 15-hydroperoxy PG, firstly because 15-hydroperoxy PGE_2 has a contractile activity on rabbit aortic strip and secondly the product(s) of the spontaneous decay of Prostacyclin then bio-assayed did not behave like PGE_2, $PGF_{2\alpha}$, or PGD_2. As Prostacyclin has an anti-aggregatory activity it cannot be identical to Thromboxane A_2 or B_2 as they are pro-aggregatory substances.

Further studies have shown the Prostacyclin has the chemical structure shown in formula (I) (R is hydrogen) (see Johnson et al., Prostaglandins, 12/6, 915–928. 1976).

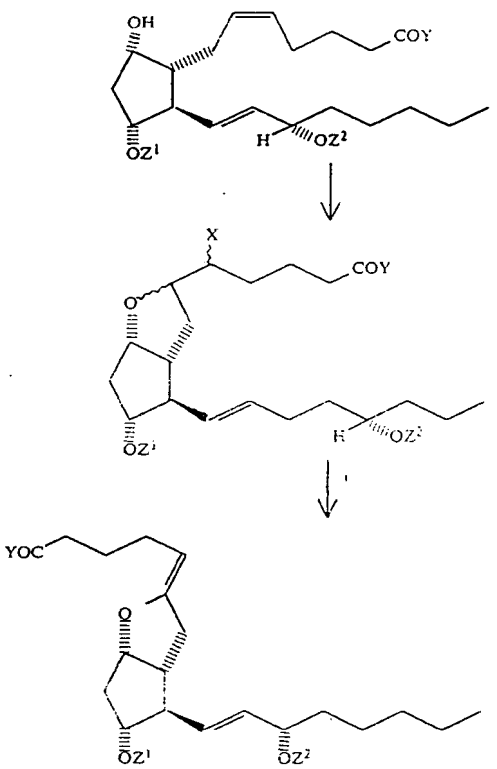


(I)

By the present invention we provide the compounds of formula (I) wherein R is hydrogen or a pharmacologically acceptable cation (hereinafter referred to as Prostacyclin and salts thereof). Salts of Prostacyclin include alkali metal salts, alkaline earth metal salts and salts of organic bases.

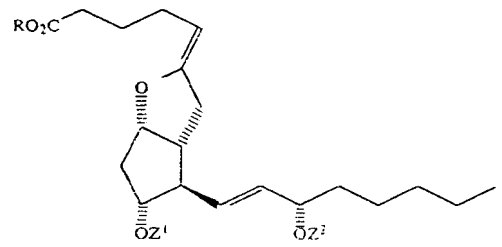
Salts of Prostacyclin may be synthesised from a compound of formula (II) wherein either or both of Z^1 and Z^2 is hydrogen or a blocking group such as acyl, or trialkylsilyl (for example trimethylsilyl). Oxidative attack by iodine or potassium triiodide in the presence of a metal bicarbonate at the 5,6-double bond of a compound of formula (II) with simultaneous or subsequent cyclisation involving the 9-hydroxy group produces a compound of formula (III). Upon treatment with a suitable base such as an organic base or a metal alkoxide,

a compound of formula (III) may then be dehydrohalogenated, resulting in the introduction of a 5,6-double bond. This reaction sequence is illustrated in the following reaction scheme:



wherein Y is OH, NHR^1 or OR^1, R^1 being alkyl of 1 to 4 carbon atoms or a cation; X is iodo or bromo; and Z^1 and Z^2 are as defined above.

Included in compounds of formula (IV) are compounds of formula



wherein R is hydrogen or a pharmacologically acceptable cation and each of Z^1 and Z^2 is hydrogen or a blocking group.

When Z and/or Z^2 in formulae (II), (III) and (IV) are blocking groups, the resulting blocked derivatives of the compounds of formula (I) may be converted to the corresponding compounds of formula (I) by methods known in the art, for example base hydrolysis.

Prostacyclin salts may be prepared by treating an ester thereof (formula (I): R is alkyl) for example the methyl ester, with a strong base such as sodium hydroxide in a suitable solvent and lyophilising the resulting reaction mixture. Prostacyclin itself may be conveniently prepared by base hydrolysis of its corresponding esters or amides in the presence of an equivalent amount of a caustic alkali in an aqueous alcohol or an aqueous tetrahydrofuran medium, and extracted into an organic solvent at low temperature.

Prostacyclin and its salts are useful as intermediates in the synthesis of prostaglandin analogues, and exhibit a potent anti-aggregatory action on blood plates, and therefore have a particular utility in the treatment and/or prophylaxis of mammals as anti-thrombotic agents.

They are also useful in mammals, including man, to reduce and control excessive gastric secretion, thereby reducing or avoiding gastrointestinal ulcer formation, and accelerating the healing of such ulcers and lesions already present in the gastrointestinal tract.

Prostacyclin and its salts further exhibit vasodilatory action on blood vessels and therefore have a particular utility as anti-hypertensives for the treatment of high blood pressure in mammals, including man. Platelets can be assimilated into the vascular endothelium or even incorporated into endothelial cells. Biochemical co-operation between platelets and vascular endothelium in the generation of Prostacyclin contributes to the repair of vascular endothelium, and Prostacyclin and its salts have a further utility in the promotion of wound healing in mammals, including man.

Prostacyclin and its salts may be used whenever it is desired to inhibit platelet aggregation, to reduce the adhesive character of platelets, and to treat or prevent the formation of thrombi in mammals, including man. For example, they may be used in the treatment and prevention of myocardial infarcts, in the treatment of peripheral vascular disease to treat and prevent post-operative thrombosis, to promote patency of vascular grafts following surgery, and to treat complications of arteriosclerosis and conditions such as atherosclerosis, blood clotting defects due to lipemia, and other clinical conditions in which the underlying etiology is associated with lipid imbalance or hyperlipidemia.

They may also be used as additives to blood, blood products, blood substitutes, and other fluids which are used in artificial extra-corporeal circulation and perfusion of isolated body portions, e.g., limbs and organs, whether attached to the original body, detached and being preserved or prepared for transplant, or attached to a new body. During these circulations and perfusions, aggregated platelets tend to block the blood vessels and portions of the circulation apparatus. This blocking is avoided by the presence of Prostacyclin. For this purpose, Prostacyclin or its salts may be added generally or in single or multiple portions to the circulating blood, to the blood of the donor animal, to the perfused body portion, attached or detached to the recipient, or to two or all of those at a total steady state dose of 0.001 to 10 mg., per liter of circulating fluid. It is especially useful to use Prostacyclin in laboratory animals e.g. cats, dogs, rabbits, monkeys and rats, for these purposes in order to develop new methods and techniques for organ and limb transplants.

The amount of prostacyclin or a salt thereof required (hereinafter referred to as the active ingredient) for therapeutic effect will vary with the route of administration. In general a suitable dose for a mammal will lie in the range of 0.01 to 200 mg. per kilogram bodyweight, conveniently 0.01 to 10 mg per kilogram.

While it is possible for the active ingredient to be administered as the raw material it is preferable to pres-

ent it as a pharmaceutical formulation. Such formulations are preferably non-aqueous and non-hydroxylic in nature, but alkaline aqueous solutions may be used. Unit doses of a formulation contain between 0.5 mg. and 1.5 g of the active ingredient.

Such formulations, both for veterinary and for human medical use, of the present invention comprise the active ingredient, as above defined, together with one or more acceptable carriers therefor and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The formulations include those suitable for parenteral (including subcutaneous, intramuscular and intravenous) administration which must of course be sterile.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

According to the present invention there are therefore provided:

(a) the compounds of formula (I) and derivatives thereof blocked in the 11- and 15-positions;

(b) the preparation of Prostacyclin comprising:

(i) incubation of microsomes of fresh mammalian tissue with a prostaglandin endoperoxide, and the extraction of prostacyclin from the incubation mixture into an organic solvent; or

(ii) its synthesis as hereinbefore described;

(c) Prostacyclin when obtained by the process described in paragraph (b);

(d) a pharmaceutical formulation containing Prostacyclin;

(e) the preparation of such pharmaceutical formulations;

(f) method for the treatment of prophylaxis of thrombosis in a mammal or mammalian tissues, including man, comprising the administration of a non-toxic, prophylactic anti-thrombotic amount of a compound of formula (I).

(g) method for inducing vasodilation in a mammal, including man, comprising the administration of a non-toxic, vasodilatory amount of a compound of formula (I);

(h) method for the prophylaxis or treatment of gastric lesions in a mammal, including man comprising the administration of a non-toxic, prophylactic or therapeutic amount of a compound of formula (I);

(i) (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin $F_{1\alpha}$ and its salts

(j) method for the promotion of wound healing in a mammal, including man, comprising the administration of a non-toxic wound-treatment amount of a compound of formula (I).

The following examples are provided by way of an illustration of the present invention and should in no way be construed as constituting a limitation thereof.

EXAMPLE 1

Preparation of Prostacyclin

Pig aortas were stripped of adventitia, snap frozen in liquid nitrogen, crushed into a fine powder, resuspended in 0.05 M Tris buffer (pH 7.5) (1:4, w:v) and homogenised at highspeed in a Polytron (KIMENATIC, LUCERNE, SWITZERLAND) homogenizer. The homogenate was centrifuged at 1000×g for 15 minutes and the resulting supernatant centrifuged again at 10,000×g for 5 minutes. The 10,000×g pellet was discarded, while the pellet obtained after centrifugation of the supernatant at 100,000 g for 60 minutes was resuspended in deionized water and lyophilized. An average yield of 150 mg. of aortic microsomal powder (51% protein) per 100 g. of aortic tissue was obtained.

Aortic microsomal powder (5 mg) was incubated with 1 μg of prostalgandin endoperoxide (PGG_2 or PGH_2) in 0.05 M Tris buffer (pH 7.5) (1 ml.) for 2 minutes at 22° C. Enzyme activity was estimated by direct bioassay of the incubation mixture. After incubation of 2 minutes at 22° C. all prostaglandin endoperoxide activity was lost indicated by a lack of contraction of rabbit aorta strips when assayed by the cascade superfusion technique of Vane (Br. J. Pharmacy. 23, 369–373, 1964 indicating 100% conversion of PGG_2 or PGH_2.

The Prostacyclin was extracted by the addition of cold dry diethyl ether (1 ml) to the incubation mixture which also stopped the enzymic reaction. The Prostacyclin entered the ether phase, which was subsequently separated from the aqueous phase. The ether was evaporated by bubbling nitrogen through it leaving the Prostacyclin which was either dissolved in ice-cold 0.05 M Tris buffer (0.5–1 ml.) and immediately used for platelet aggregation studies or was dissolved in anhydrous acetone (1 ml.) and stored at −20° C. for future use.

The anti-aggregatory activity of the extracted Prostacyclin disappeared on bioling (15 seconds) or on standing at 22° C. for 20 minutes. The anti-aggregatory activity of Prostacyclin could be preserved for several days by dissolving the substance in dry acetone and storing at −20° C

By using rabbit aortas in a similar experiment the same results were obtained.

EXAMPLE 2

Aggregation of platelets in 1 ml. of fresh human platelet rich plasma (PRP) was monitored in a Born aggregometer. An immediate anti-aggregatory effect of the fresh reaction mixture of Example 1 of aortic microsomes with PGH_2 or PGG_2 was observed. The lowest anti-aggregatory concentration was obtained with samples containing 0.5–5 ng. Prostacyclin/ml.

This activity disappeared after leaving the incubation mixture for 20 minutes at 22° C. or by bioling for 15 seconds. Aortic microsomes alone (50 μg/ml) could induce aggregation in some PRP. The products of spontaneous degradation of PGG_2 (100 ng/ml) had no anti-aggregatory activity.

Diethyl ether extracts of Prostacyclin also inhibited platelet aggregation induced by arachidonic acid and PGG_2. The effective anti-aggregatory concentration of Prostacyclin after storate in ether was from 1 to 10 ng/ml

EXAMPLE 3

Prostacyclin was injected intravenously and intra-aortically into normotensive anaethetised rats and the blood pressure and heart rate recorded. Results showed Prostacyclin to be a powerful vasodepressor having 5 times the potency of PGE_2.

EXAMPLE 4

Prostacyclin was found to relax spirally cut strips of coronary artery from the ox. The effect is dose dependent and relaxation can be seen with doses as low as 20 nanograms per 5 ml bath. In isolated hearts from rabbits perfused at constant flow rate by the Langendorf technique, Prostacyclin produced coronary vasodilation.

Prostacyclin relaxed strips of coeliac and mesenteric arteries, but was less potent than PGE_2.

EXAMPLE 5

Prostacyclin was shown to inhibit the formation of gastric lesions induced in rats by indomethacin at doses from 62.5 to 250 μg/kg. upon subcutaneous injection

EXAMPLE 6

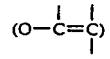
A stirred solution of $PGF_{2\alpha}$methyl ester (50 mg) in ether (1 ml) was treated with sodium bicarbonate (115.0 mg; 10 molecular equivalents) and water (1 ml) and then dropwise during 2 hours with aqueous potassium triiodide (0.7 molar; 0.261 ml). After stirring overnight, the reaction mixture was shaken with ether and aqueous sodium thiosulphate; the etheral phase was separated, washed with water, dried with magnesium sulphate, and evaporated to leave a yellow gum of 5ξ-iodo-9-deoxy-6ξ, 9α-epoxyprostaglandin $F_{1\alpha}$methyl ester.

A solution of 5ξ-iodo-9-deoxy-6ξ, 9α-epoxyprostaglandin $F_{1\alpha}$methyl ester (100 mg.) in methanolic sodium methoxide prepared from sodium (46 mg.) and dry methanol (0.70 ml.) was set aside under dry nitrogen for 5 hours, then freed from solvent in high vacuum. The residual amorphous solid was washed with benzene, set aside in the air overnight, and stirred with N aqueous sodium hydroxide (0.5 ml.) to give a suspension of colourless fine needles. The crystals were collected, washed with a few drops of N aqueous sodium hydroxide. and dried in the air to give the sodium salt of 9-deoxy-6,9α-epoxy-Δ^5-prostaglandin $F_{1\alpha}$. The inhibition of arachiodonic acid-induced aggregation of human platelets at a concentration of 0.2 ng./ml. by this salt and its instability in water at acid pH, together with further evidence, is compatible with assignation of the configuration (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxy-prostaglandin $F_{1\alpha}$sodium salt.

The high-resolution ^{13}C n.m.r. spectrum of a solution of the crystals in dimethyl sulphoxide-d_6 showed the expected 20 resonances whose chemical shifts were entirely consistent with the chemical structure established for Prostacyclin. No impurity peaks were detected.

EXAMPLE 7

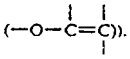
5ξ-Iodo-9-deoxy-6ξ, 9α-epoxyprostaglandin $F_{1\alpha}$-methyl ester (500 mg) was stirred with methanolic NaOMe prepared from Na (0.23 g., 10 equivs.) and MeOH (3.5 ml.) under N_2 at room temperature overnight; 1N aq. NaOH (2.5 ml.) was added to the yellow reaction solution to bring about hydrolysis of the ester moiety and, after 2 hours, the methanol was evaporated in vacuo at room temperature. The residual aqueous solution gave rise spontaneously to a mass of colourless fine needles of the desired sodium salt (formula (I): R=Na) which was cooled (0°), collected, washed sparingly with 1N aq. NaOH, air-dried, and stored in a stoppered tube; this salt (383 mg.) had max (KBr disc) 1692 cm^{-1}



and twenty ^{13}C resonances only were observed (at 182.7 (C-1), 158.2 (C-6), 140.0 and 134.3 (C-13, 14), 100.7(C-5), 87.5(C-15), 80.6 and 75.5(C-9, 11), 58.0(C-12), 49.0, 45.8, 42.4, 41.9, 37.5, 35.8 (C-18), 31.6., 29.9, 29.3, 26.7(C-19), and 18.4(C-20) ppm from TMS in DMSO-d_6). The product, sodium (5Z)- 5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin $F_{1\alpha}$(syn. sodium prostacyclin), thus obtained completed inhibited arachidonic acid-induced platelet aggregation (human platelet-rich plasma) at 1 ng./ml. and its profile of biological activity on the rabbit aorta, rabbit coeliac artery, rat stomach strip and rat colon conformed with that of sodium prostacyclin obtained by biosynthesis. After air-drying, the salt has a surface coating of sodium carbonate (ca 3.5% by weight) which protects the vinyl ether moiety against carbondioxide catalysed hydrolysis.

EXAMPLE 8

5ξ-Iodo-9-deoxy-6ξ,9α-epoxyprostaglandin $F_{1\alpha}$-methyl ester was treated with 1,5-diazabicyclo-5-nonene (DBN) at room temperature in the absence of a solvent for a few hours. The DBN and hydrogen iodide were conveniently removed by adsorption on to a column of SiO_2, prepared from a suspension of SiO_2 in EtOAc/Et_3N 50:1, and the vinyl ether was eluted with the same solvent system. I.R. spectroscopy (thin film, ν_{max} 1738 (CO_2Me) and 1696 cm^{-1}



^1H n.m.r. in C_6D_6-Et_3N, 19:1 (δ4.22, triplet of triplets[12], J6.9 and 1.0 Hz (C-5 vinyl proton)), and ^{13}C n.m.r. in C_6D_6-Et_3N, 19:1 (distinctive features were resonances at 159.8 (C-1), 155.8 (C-6), 137.2 and 130.6 (C-13,14), 95.3 (C-5), 84.1 (C-15) 77.3 and 72.2 (C-9,11) and 51.1. (Me ester)p.p.m. from TMS).

The vinyl ether (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxyprostaglandin methyl ester, was hydrolysed with aqueous sodium hydroxide to give sodium prostacyclin (formula (I): R=sodium).

What we claim is:

1. Prostacyclin substantially free from other biological material.

2. The method of treating thrombosis in a mammal which comprises administering an effective antithrombotic portion of prostacyclin to said mammal in need thereof.

3. The method of inhibiting the aggregation of blood platelets in a mammal which comprises administering an effective platelet antiaggregation amount of prostacyclin to said mammal.

4. A method of preventing thrombosis in a mammal in need thereof which comprises administering an effective thrombosis prevention amount of prostacyclin to said mammal.

5. A method for the prevention or treatment of gastric lessions in a mammal comprising the administration of a non-toxic prevention or treatment amount of prostacylin to said mammal.

6. A method for the treatment of a wound in a mammal which comprises administering to said mammal a non-toxic wound treatment amount of prostacylin.

7. A method for producing a vasodilatory action in the blood vessels of a mammal in need thereof comprising the administration of an effective vasodilatory amount of prostacyclin to said mammal.

8. The method of preparing (5-Z)-5,6-Didehydro-9-deoxy-6,9α-epoxyprostaglandin $F_{1\alpha}$ which comprises incubating microsomes of mammalian tissue with a prostaglandin endoperoxide.

9. The method of claim 8 in which the microsomes are aortic microsomes and the prostaglandin endoperoxides are PGG_2 or PGH_2.

* * * * *

EXHIBIT 11

U.S. PATENT 4,338,325

United States Patent [19]

Johnson et al.

[11] **4,338,325**

[45] **Jul. 6, 1982**

[54] **PGI_2 PHARMACOLOGICALLY ACCEPTABLE SALTS**

[75] Inventors: **Roy A. Johnson, Kalamazoo; Frank H. Lincoln, Portage; John E. Pike, Kalamazoo, all of Mich.**

[73] Assignee: **The Upjohn Company, Kalamazoo, Mich.**

[21] Appl. No.: 200,690

[22] Filed: Oct. 27, 1980

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 819,940, Jul. 28, 1977, which is a continuation-in-part of Ser. No. 725,550, Sep. 22, 1976, abandoned, which is a continuation-in-part of Ser. No. 716,770, Aug. 23, 1976, abandoned.

[51] Int. Cl.³ **A61K 31/557; C07D 307/935**

[52] U.S. Cl. **424/285; 549/465**

[58] Field of Search 260/346.22, 346.73; 542/421; 424/285

[56] References Cited

U.S. PATENT DOCUMENTS

3,598,858 8/1971 Bergstrom et al. 562/503

OTHER PUBLICATIONS

Moncada et al., Prostaglandins, vol. 12, pp. 658–713, (1976).

Primary Examiner—Henry R. Jiles
Assistant Examiner—Bernard Dentz
Attorney, Agent, or Firm—Robert A. Armitage

[57] ABSTRACT

The present invention relates to PGI_2 pharmacologically acceptable salts, having pharmacological activity. Particularly, the compounds described herein are useful as platelet aggregation inhibitors.

6 Claims, No Drawings

PGI_2 PHARMACOLOGICALLY ACCEPTABLE SALTS

DESCRIPTION

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of co-pending application Ser. No. 819,940, filed July 28, 1977; which is a continuation-in-part of Ser. No. 725,550, filed Sept. 22, 1976, now abandoned; which was a continuation-in-part of Ser. No. 716,770, filed Aug. 23, 1976, now abandoned.

BACKGROUND OF THE INVENTION

The present invention relates to novel compositions of matter which are derivatives of prostacyclin or PGI_2. The chemical name for prostacyclin is (5Z)-5,6-didehydro-9-deoxy-6,9α-epoxy-$PGF_1\alpha$. Specifically the present invention relates to PGI_2 pharmacologically acceptable salts.

Prostacyclin itself was first reported as "PGX" by Moncada and his co-workers. See Moncada, et al., Prostaglandins 12:658–713 (1976). Prostacyclin is a circulating hormone in the arterial circulation of mammals. See "Prostacyclin As A Circulating Hormone", Nature 273:767–768 (June 29, 1978) and Gryceweski, R. J., et al., "Generation of Prostacyclin By Lungs In Vivo And Its Release Into Arterial Circulation," Nature 273:765–767 (June 29, 1978).

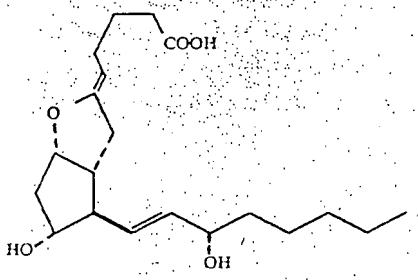
PRIOR ART

Subsequent to any invention described herein the existence of prostacyclin as a naturally-occurring composition of matter was reported in the aforementioned references.

SUMMARY OF THE INVENTION

The present invention relates to

a composition of matter consisting essentially of a pharmacologically acceptable salt of a compound of formula I

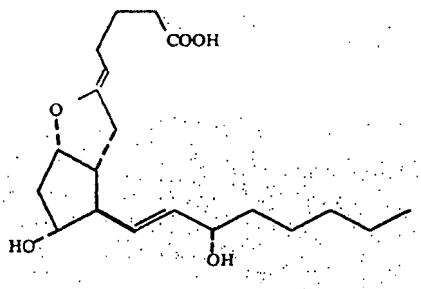


I

and

a parenteral pharmaceutical composition characterized in being prepared from

(1) a free flowing powder form of the sodium salt of PGI_2, a compound of formula I



I

and

(2) a conventional pharmaceutical diluent for parenteral formulations.

Compounds in accordance with the present invention are prepared by chemical methods and have pharmacological uses. Accordingly, compounds in accordance with the present invention are useful for a variety of pharmacological purposes. Accordingly, the compounds in accordance with the present invention are especially useful whenever it is desired to inhibit platelet aggregation, to reduce the adhesive character of platelets, and to remove or prevent the formation of thrombi in mammals, including man. For example, these compounds are useful in the treatment and prevention of myocardial infarcts, to treat and prevent post-operative thrombosis, to promote patency of vascular drafts following surgery, and to treat conditions such as atherosclerosis, arteriosclerosis, blood clotting defects due to lipemia, and other clinical conditions in which the underlying etiology is associated with lipid imbalance or hyperlipidemia. Other in vivo applications include geriatric patients to prevent cerebral ischemic attacks and long-term prophylaxis following myocardial infarcts and strokes. For these purposes, the compounds are administered systemically, e.g., intravenously, subcutaneously, intramuscularly, and in the form of sterile implants for prolonged action. For rapid response, especially in emergency situations, the intravenous route of administration is preferred. Dosages in the range about 0.01 to about 10 mg/kg of body weight per day are used, the exact dose depending on the age, weight, and condition of the patient or animal, and on the frequency and route of administration.

The addition of these compounds to whole blood provides in vitro applications, such as storage of whole blood to be used in heart-lung machines. Additionally, whole blood containing these compounds can be circulated through limbs and organs, e.g., heart and kidneys, whether attached to the original body, detached and being preserved or prepared for transplant, or attached to a new body. Blocking of aggregated platelets is avoided by the presence of these compounds. For this purpose, the compound is added gradually or in single or multiple portions to the circulating blood, to the blood of the donor person or animal, to the perfused whole body, attached or detached, to the recipient, or to two or all of those at a total steady state dose of about 0.001–1.0 μg/ml of whole blood. These compounds are also useful in preparing platelet-rich concentrates from blood for use in treating thrombocytopenia or in che-

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention especially provides for the preparation of compositions of matter consisting essentially of pharmacologically acceptable salts of prostacyclin. Most conveniently, these pharmacologically acceptable salts of prostacyclin are prepared from the corresponding methyl ester, PGI_2 methyl ester or (5Z)-9-deoxy-6,9α-epoxy-5,6-didehydro-PGF_1, methyl ester. The preparation of the methyl ester starting material is described in U.S. Pat. No. 4,158,667, incorporated here by reference (e.g., Example 7 therein). From the crystalline form of this methyl ester described in U.S. Pat. No. 4,158,667, compositions of matter consisting essentially of pharmacologically acceptable salts of prostacyclin are prepared in accordance with the following examples:

EXAMPLE 1

(5Z)-9-Deoxy-6,9α-epoxy-Δ^5-PGF_1, sodium salt.

A mixture of 0.030 g (5Z)-9-deoxy-6,9α-epoxy-Δ^5-PGF_1, methyl ester in 5 ml of methanol is treated with 9 ml of 0.01 N NaOH and stirred at about 250° C. for 72 hr. The solution is then concentrated, diluted with 5 ml of water, frozen at about −75° C. and lyophilized overnight. The title compound is obtained as a white free-flowing powder having infrared absorption at 3320, 1693, 1555, and 1470 cm^{-1}.

The procedure above is repeated using larger quantities. From 0.150 g of the enol ether methyl ester there is obtained 0.155 g of the title compound as a white free-flowing powder. A sample of the material dissolved in methanol-water shows practically no mobility by TLC on silica gel plates in acetone-dichloromethane (3:7), compared with the starting material which has R_f 0.45 (TLC on silica gel in acetone-dichloromethane (3:7) using plates pretreated in triethylamine-(5%)-dichloromethane).

Following the procedure of Example 1, but replacing sodium hydroxide with excess sodium carbonate suspended in water, there is obtained the title compound after 72 hr at about 25° C. The product is precipitated with acetonitrile.

EXAMPLE 2

(5Z)-9-Deoxy-6,9α-epoxy-Δ^5-PGF_1, benzyltrimethylammonium salt.

A solution of 0.25 g of (5Z)-9-deoxy-6,9α-epoxy-Δ^5-PGF_1, methyl ester in 5 ml of methanol is treated with one ml of water and 0.5 ml of methanolic solution (40%) of benzyltrimethylammonium hydroxide. The mixture is left standing at about 25° C. for 22 hr and then concentrated to one-fourth volume. The concentrate is made up with water to a volume of 25 ml, frozen with Dry Ice and lyophilized to yield the title compound, a solid, having infrared absorption at 3375, 1700, 1570, 1395, 1125, 1090, 1035, 975, 895, 780, 725, and 705 cm^{-1}.

EXAMPLE 3

(5Z)-9-Deoxy-6,9α-epoxy-Δ^5-PGF_1, tetramethylammonium salt

A solution of 0.25 g of (5Z)-9-Deoxy-6,9α-epoxy-Δ^5-PGF_1, methyl ester in 5 ml of methanol is treated with one ml of an aqueous solution (10%) of tetramethylammonium hydroxide, thereafter following the procedure of Example 2, to obtain the title compound, a solid, having infrared absorption at 3375, 1695, 1570, 1495, 1395, 1125, 1090, 1035, 970, and 955 cm^{-1}.

Accordingly, there is provided by the present invention novel compositions of matter consisting essentially of a single, pharmacologically acceptable salt of prostacyclin.

Accordingly, the present compositions of matter consisting essentially of a pharmacologically acceptable salt of prostacyclin are surprisingly and unexpectedly more useful than preparations of prostacyclin heretofore obtainable from biological sources in that the novel compositions of matter are

(1) highly chemically homogeneous

(2) more readily sterilized for parenteral administration,

(3) more conveniently and elegantly formulated as pharmaceutical entities, and

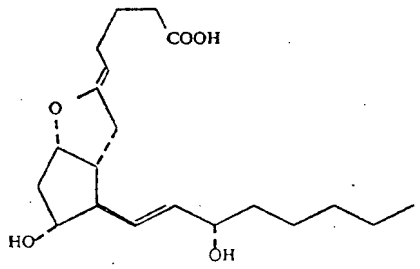
(4) more efficacious in obtaining prostaglandin-like biological effects.

Thus, the present invention provides compositions of matter surprisingly and unexpectedly more useful than forms of prostacyclin known prior to the present invention.

We claim:

1. A composition of matter consisting essentially of a pharmacologically acceptable salt of a compound of formula I

I



2. A composition according to claim 1 wherein said pharmacologically acceptable salt is the benzyltrimethylammonium salt.

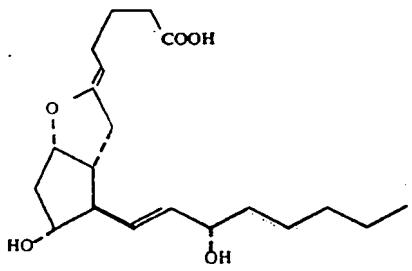
3. A composition according to claim 1 wherein said pharmacologically acceptable salt is the tetramethylammonium salt.

4. A composition according to claim 1 wherein said pharmacologically acceptable salt is the sodium salt.

5. A composition according to claim 4 in a free flowing powder form.

6. A parenteral pharmaceutical composition for inhibition of platelet aggregation characterized in being prepared from

(1) a free flowing powder form of the sodium salt of [illegible] of claim 1



and

(2) a conventional pharmaceutical diluent for parenteral formulations, such that the sodium salt of PGI_2 is present in said composition at a concentration sufficient to inhibit platelet aggregation upon administration of a said composition in a predetermined volume or at a predetermined rate.

* * * * *

EXHIBIT 12

TERMINAL DISCLAIMER
FOR
U.S. 4,883,812

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Salvador Moncada GROUP : 121

SER. NO. : 07/237,987 EXAMINER: Dentz, B.

FILED : August 29, 1988

FOR : ETHERS

Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A PRIOR PATENT

Your petitioner, BURROUGHS WELLCOME CO. residing at Research Triangle Park in the State of North Carolina represents that it is the owner of said application Serial No. 07/237,987, filed on the 29th day of October, 1988 for ETHERS. ITS ownership by way of an assignment is recorded in parent application Serial Number 795,524, filed May 10, 1977, now U.S. Patent 4,539,333 of which this application is a continuation, at Reel 3479, Frames 683-686, recorded on December 7, 1977. Your petitioner hereby disclaims the terminal part of any patent granted on the above-identified application, which would extend beyond the expiration date of the full statutory term as presently shortened by any terminal disclaimer of Patent No. 4,539,333, and hereby agrees that any patent so granted on the above-identified application shall be enforceable only for and during such period that the legal title to United States Patent No. 4,539,333 this agreement to run with any patent granted on the above-identified application and to be binding the grantee, its successors or assigns.

Petitioner does not disclaim any terminal part of any patent granted on the above-identified application prior to the expiration date of the full statutory term as presently shortened by any terminal disclaimer of Patent No. 4,539,333, in the event that it later expires for failure to pay a maintenance fee, is held unenforceable, is found invalid, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321(a), has all claims cancelled by a reexamination certificate, or is otherwise terminated prior to the expiration of its statutory term as presently shortened by any terminal disclaimer, except for the separation of legal title stated above.

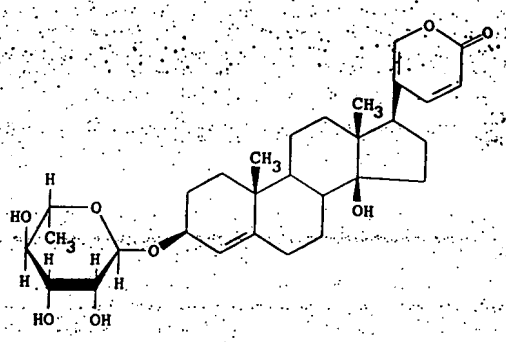
BURROUGHS WELLCOME CO.

By: David A. Powell 6/13/89
Its Vice President

EXHIBIT 13

ITEM 7890 MERCK INDEX
PROSTACYCLIN

20, 1 (1970). Metabolic studies: Davis *et al., Arch. Int. Pharmacodyn.* 177, 231 (1969); Nakano *et al., ibid.* 183, 199 (1970). Clinical studies: Several authors, *Minerva Med.* 58, 4243-4322 (1967).



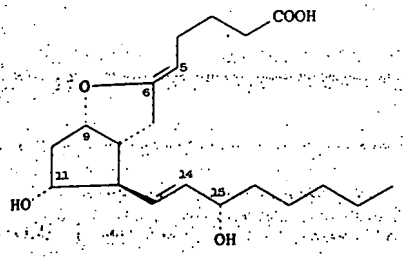
Prisms from methanol, mp 219-222°, $[\alpha]_D^{20}$ −91.5° (CH_3OH). LD_{50} orally in male, female rats: 56, 76 mg/kg. E. I. Goldenthal, *Toxicol. Appl. Pharmacol.* 18, 185 (1971).

Proscillaridin-4'-methyl ether, $C_{31}H_{44}O_8$, *meproscillarin, Clift.* mp 213-217°. $[\alpha]_D^{20}$ −94° (CH_3OH). uv max (CH_3OH): 297 nm (log ε 3.79), (1*N* KOH/CH_3OH): 355 nm (log ε 4.65). Sol in methanol, ethanol, THF, dioxane; slightly sol in $CHCl_3$, CH_2Cl_2, acetone; insol in water, nonpolar organics. Series of articles on prepn, pharmacology, toxicology, pharmacokinetics, metabolism: *Arzneimittel-Forsch.* 28, 493-573 (1978).

THERAP CAT: Cardiotonic.

7890. Prostacyclin. *(5Z,9α,11α,13E,15S)-6,9-Epoxy-11,15-dihydroxyprosta-5,13-dien-1-oic acid;* (5Z)-9-deoxy-6,9α-epoxy-Δ^5-$PGF_{1\alpha}$; epoprostenol; prostaglandin I_2; prostaglandin X; PGI_2; PGX; U-53217. $C_{20}H_{32}O_5$; mol wt 352.48. C 68.15%, H 9.15%, O 22.70%. A prostaglandin produced by enzymatic transformation of prostaglandin endoperoxides (*PGG_2*, *PGH_2*), which dilates blood vessels and is approximately 30 times more potent than prostaglandin E_1, *q.v.*, in inhibiting platelet aggregation. Evidence for its occurrence during biosynthetic conversion of arachidonic acid by rat stomach homogenates: C. Pace-Asciak, L. S. Wolfe, *Biochemistry* 10, 3657 (1971). Isoln from microsomes of pig and rabbit aorta by J. R. Vane and co-workers: S. Moncada *et al., Nature* 263, 663 (1976). PGI_2 is also synthesized in bovine coronary arteries as well as human arteries and veins: *eidem, Lancet* 1, 18 (1977); G. J. Dusting *et al., Prostaglandins* 13, 3 (1977); by cultured human and bovine endothelial cells: B. B. Weksler *et al., Proc. Nat. Acad. Sci. USA* 74, 3922 (1977); by pig aortic endothelial cells: D. E. MacIntyre *et al., Nature* 271, 549 (1978). It has been suggested that endoperoxides released by platelets can be converted to PGI_2 by vascular tissue and that a balance between formation of PGI_2 and release of thromboxane A_2, *q.v.*, which induces platelet aggregation, controls the formation of thrombi in blood vessels. It has also been postulated that PGI_2 acts to stimulate platelet adenylate cyclase and to prevent the action of thrombi on phospholipid breakdown as well as platelet aggregation. Structure: R. A. Johnson *et al., Prostaglandins* 12, 915 (1976). Synthesis: E. J. Corey *et al., J. Am. Chem. Soc.* 99, 3006 (1977); of sodium salt and stereochemistry: R. A. Johnson *et al., ibid.* 4182. Additional syntheses: I. Tomoskozi *et al., Tetrahedron Letters* 1977, 2627; N. Whittaker, *ibid.* 2805; K. Nicolaou, *Chem. Commun.* 1977, 630. Synthesis of the 5*E*-isomer: E. J. Corey *et al., Tetrahedron Letters* 1977, 3529. Chemical stability in aq solns: M. J. Cho, M. A. Allen, *Prostaglandins* 15, 943 (1978).

Biosynthetic study: V. Tomasi *et al., Nature* 273, 670 (1978). Biological properties: R. J. Gryglewski *et al., Prostaglandins* 12, 685 (1976). Preliminary clinical study: A. E. S. Gimson *et al., Lancet* 1, 173 (1980). Antimetastatic effects: K. V. Honn *et al., Science* 212, 1270 (1981); *eidem, ibid.* 217 542 (1982). Preliminary study of effect of PGX infusion in patients with acute myocardial infarction: O. Edhag *et al., N. Engl. J. Med.* 308, 1032 (1983). Review of biological properties: S. Moncada, J. R. Vane, *Clin. Sci.* 61, 369-372 (1981); of therapeutic potential: *eidem, Advan. Pharmacol. Ther.* 4, 215-233 (1982); of physiological role: J. R. Vane *et al., Int. Rev. Exp. Pathol.* 23, 161-207 (1982). General reviews: S. Moncada, J. R. Vane, *Fed. Proc.* 38, 66-71 (1979); J. C. McGiff, *Ann. Rev. Pharmacol. Toxicol.* 21, 479-509 (1981); S. Moncada *et al., Advan. Pharmacol. Ther.* 6, 39-47 (1982). Books: *Prostacyclin*, J. R. Vane, S. Bergstrom, Eds. (Raven Press, New York, 1979) 453 pp; *Prostaglandins in Cardiovascular and Renal Function*, A. Scriabine *et al.*, Eds. (Spectrum Publications, New York, 1980) 498 pp.

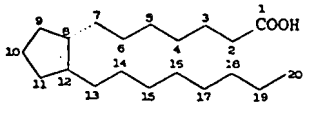


Chemically unstable in aq soln. Hydrolyzes to 6-oxo-$PGF_{1\alpha}$. Half-life at 4° is approx 14.5 min when total phosphate is 0.165 *M*. Anti-aggregating activity disappears within 0.25 min on boiling or within 10 min at 37°.

Sodium salt, $C_{20}H_{31}NaO_5$, *U-53217A, Cyclo-Prostin, Flolan.* Hygroscopic, free-flowing white powder. Stable for 2 months if kept dry at −30°.

THERAP CAT: Platelet aggregation inhibitor.

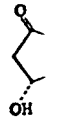
7891. Prostaglandin(s). A family of biologically potent lipid acids first discovered in seminal fluid and extracts of accessory genital glands of man and sheep: von Euler, *Arch. Exp. Pathol. Pharmakol.* 175, 78 (1934); *Klin. Wochenschr.* 14, 1182 (1935). Isoln: Bergstrom, Sjovall, U.S. pats. 3,069,322 and 3,598,858 (1962, 1971); Samuelsson, *J. Biol. Chem.* 238, 3229 (1963). Also found in lower concns in other organs: *idem, Biochim. Biophys. Acta* 84, 707 (1964). The single non-mammalian source of prostaglandin intermediates, or *syntons*, is the gorgonian sea whip or sea fan, *Plexaura homomalla:* Weinheimer, Spraggins, *Tetrahedron Letters* 1969, 5185; Schneider *et al., J. Am. Chem. Soc.* 94, 2122 (1972). Prostaglandins are named as derivatives of *prostanoic acid.* Prostaglandins are divided into the types E, F, A, B, C, and D based on functions in the cyclopentane ring. Numerical subscripts refer to the number of unsaturations in the side chains; α or β subscripts refer to the configuration of substituents in the ring. Six naturally occurring prostaglandins, E_1, E_2, E_3, $F_{1\alpha}$, $F_{2\alpha}$, $F_{3\alpha}$, are considered primary in that no one is derived from another in the living organism. First structural and stereochemical elucidations: Bergstrom *et al., Acta Chem. Scand.* 16, 501 (1962); *eidem, J. Biol. Chem* 238, 3555 (1963). Absolute config: Nugteren *et al., Nature* 212, 38 (1966). First total synthesis of racemic PGF_1 and $PGF_{1\alpha}$: Corey *et al., J. Am. Chem. Soc.* 90, 3245 (1968). Review of synthetic studies: Pike, *Fortschr. Chem. Org. Naturst.* 28, 313 (1970); Axen *et al.*, in *The Total Synthesis of Natural Products*, vol. 1, J. ApSimon, Ed. (Wiley-Interscience, New York, 1973) pp 81-143; Clarkson in *Progress in Organic Chemistry*, vol. 8, W. Carruthers, J. K. Sutherland, Eds. (Wiley, New York, 1973) pp 1-28. Book: J. S. Bindra, R. Bindra, *Prostaglandin Synthesis* (Academic Press, New York, 1977).



prostanoic acid

Biosynthesis occurs by enzymatic conversion of unsaturated twenty-carbon fatty acids. Review of biosynthetic studies: Samuelsson, *Progr. Biochem. Pharmacol.* 5, 109 (1969). Review of metabolism: Samuelsson *et al., Ann. N.Y. Acad. Sci.* 180, 138 (1971). Biological activities include stimulation of smooth muscle, dilation of small arteries, bronchial dilation, lowering of t tion, of lipolysis, labor, abortion, a pressure. Implica reactions, nasal v autonomic neurotr and biochemical (1965); Weeks, *An* man, *Ann. Rev. Bi* cal activities of s *Nature* 221, 1251 (cal and preparativ tled "Prostaglandi M. Landis, W. L. 1982) 705 pp. Ge (1967); Bergstron Ramwell, Shaw, *I* Pike, *Sci. Amer.* 2 *gress in Drug Res* 1973) pp 410-487. Ramwell, Ed. (P *Prostaglandins in* Scriabine *et al.*, 1980) 498 pp; *Ca glandins*, A. Herm 472 pp. Three v *Reproduction; Pro Aspects; Prostaglan Pathological Aspect* sity Park Press, Ba

7892. Prostagl *13-en-1-oic acid; cyclopentanehepta* Liple; Minprog; P mol wt 354.49. C prostaglandin; eas extracts. Isoln fro ture: Bergstrom *e eidem, J. Biol. Che* from 8,11,14-eicos *Chim.* 85, 405 (196 *J. Am. Chem. Soc. ibid.* 5895; 91, 53 1969, 303; Taub *e* 1972, 304; Kuo *et et al., Tetrahedron Commun.* 1973, 1 (1973). Synthesis *Chem. Soc.* 91, 53 94, 3643 (1972); *Chem.* 37, 2921 (1974). Metaboli *J. Biol. Chem.* 23 Hamberg, Samuels biological activities 3, 110 (1967). Co other prostaglandi (1972). Clinical us in neonatal cardiac *taglandin Thrombo al., J. Thoracic Car et al., Circulation* 6 vasculopathy: D. 1 1846 (1981). For



Crystals from e $[\alpha]_{578}$ −6[illegible].6° (c = drated in soln at pH THERAP CAT: Vas

7893. Prostagla *droxy-9-oxoprosta-*